BIOCONTROL OF POSTHARVEST DISEASES OF SOLANACEOUS VEGETABLES

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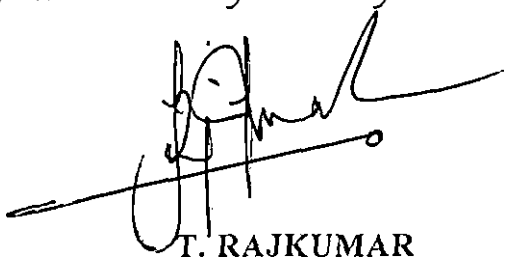
DECLARATION

I hereby declare that this thesis entitled "Biocontrol of postharvest diseases of solanaceous vegetables" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.



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INTRODUCTION

1. INTRODUCTION

Postharvest losses of fruits and vegetable due to diseases has been estimated to be 24 per cent of the harvested crop in the US and almost 50 per cent in the under developed tropical countries (USDA, 1965). In India, post harvest losses as high as 60 per cent have been recorded from some markets and such losses are more during rainy season (Mandal and Dasgupta, 1989). The chemicals used for prevention of postharvest spoilage of fruits and vegetables are gradually being phased out considering the public health and environmental pollution risk they pose. The potential for using biological control methods for the management of postharvest diseases assumes greater importance in this perspective.

There are examples of a number of postharvest diseases of peaches (Pusey *et al*, 1986; and Pusey and Wilson, 1984), cherries (Utkhede and Sholberg, 1986), apples (Janiseiwicz, 1987; 1988), pears (Janiseiwicz and Roitman, 1988), citrus (Chalutz and Wilson, 1989) and strawberries (Tronsmo and Dennis, 1977) which have been controlled by introduced antagonists. Biological control using antagonists in the postharvest environment is more advantageous since it is more amenable to human interventions than field conditions. Moreover under postharvest conditions the antagonists can be more easily targeted to where they are needed when compared to field or soil application.

The present study was taken up with the view to:

a) Isolate and identify the fungal pathogens associated with solanaceous vegetables viz. brinjal, chilli and tomato.

b) Isolate and identify the epiphytic mycoflora of the healthy fruits of brinjal, chilli and tomato.

c) *In vitro* study of the antagonism of epiphytic fungi with the major pathogens of brinjal, chilli and tomato and evaluating suitable antagonistic fungi.

d) Study the mechanism of action of antagonism.

e) Study the effect of the selected mycoparasite against the important fruit rot pathogens.

f) Test the viability and survival of the selected antagonists on different carrier materials.

g) Formulate the antagonist in the carrier material and to compare the methods of application of the formulation.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The relevant literature regarding the subject of study is presented under areas like, fungi associated with the spoilage of solanaceous vegetables, isolation of epiphytic microflora, their mode of antagonism *in vitro* and *in vivo* conditions and the formulation of antagonists. Considerable amount of work has been done on these aspects globally and to a lesser extent in India.

2.1 Incidence of various fungal diseases in solanaceous vegetables

2.1.1 Brinjal (*Solanum melongena* L.)

Pawar and Patel (1957) studied the symptom development of *Phomopsis vexans* on brinjal and recorded them as minute sunken dull purple lesions which coalesce to form large blotches. These blotches show numerous pycnidia of the fungi on it. John, (1991) recorded fungal growth in concentric rings with yellow and brown zones. The lesions then increased in size and large rotted areas were formed with many sunken pycnidia (John, 1991).

Ramakrishnan and Wilson (1968) surveyed the markets of Thiruvananthapuram and found *Rhizopus* and *Diplodia* rot on brinjal as the important ones. The *Rhizopus* rot is characterised by profuse white growth around the rotted fruit, while *Diplodia* rot showed black growth on the surface of the fruit accompanied with extensive rotting of the tissues. Alice and Pailey (1978) reported that both *Rhizopus* rot and *Diplodia* rot produced mycelial growth on the fruit surface and resulted in watery exudations.

Fusarium infection is also noted (Alice, 1969). Kaur *et al.* (1985) surveyed the markets in Punjab and concluded that about 7 - 30 per cent rot incidence in brinjal is due to *Fusarium semitectum*. Kumar *et al.* (1986) also in their market surveys in Punjab obtained *Fusarium oxysporum* and *F. moniliforme*. *Fusarium* spp. induces infection at stylar end and the rot progresses ahead rapidly.

Rotting due to *Aspergillus* species is also recorded (Alice, 1969 and Kumar *et al.*, 1986). The rots initiated as brown water soaked lesions and resulted in the complete rotting of the fruit.

Singh and Shukla (1986) recorded fruit rot caused by *Alternaria alternata* to be an important disease in India causing heavy loss of yield. The disease appeared in two phases viz., as leaf spots and fruit rots and was influenced with high relative humidity and temperature.

Rotting due to *Phytophthora* species is also recorded by Gogoi and Rathaiah (1992). They obtained two types of symptoms caused by *Phytophthora infestans* on aubergines viz. leaf blights and dry fruit rot.

2.1.2 Chilli (*Capsicum annuum* L.)

The dry rot of chilli caused by *Colletotrichum capsici* (Gupta *et al.*, 1983). Khaleeque and Khan (1991) also found it to be frequently associated with fruit rot. Sharma and Sumbali (1993) also recorded *C. capsici* from chilli.

Senappa *et al.* (1980) observed *Aspergillus* rots to be common at 28^{0}C and at relative humidity above 85 per cent. The germtubes of the pathogen were

found to enter through stomata on the fruit. Adisa (1985) from Nigeria described chilli fruit rots caused by *A. fumigatus* and *A. flavus* and accounted for 40-45 per cent spoilage during wet season.

Ekundayo and Osa (1993) studied the fungal spoilage of fresh peppers in South Africa and recorded *Botryodiplodia theobromae* and *Rhizopus oryzae* in addition to *Aspergillus flavus*. The fungi gained entry through stalk end and injuries and caused rotting and spoilage of the fruits.

2.1.3 Tomato (*Lycopersicon esculentum* Mill.)

Zitter and Wien (1984) studied the *Alternaria alternata* infection on ripe tomatoes and found the infection mainly at the stalk end and the shoulders. Lesion as big as 13 mm were recorded by them. Sharma and Sumbali (1993) conducted extensive survey at wholesale, retail and consumer levels and observed tomato rots due to fungi like *Sclerotinia sclerotiorum*, *Colletotrichum capsici*, *Alternaria alternata*.

Butler (1960) reported the loss of large quantities of tomatoes due to *Geotrichum candidum*. It is a weak parasite which colonises the broken and ruptured fruits. It causes a typical star shaped rupture after 3-4 days of infection. The growth of the fungi as a white mass can be seen along the cut surface. The spread of the above pathogen has been attributed to the common fruit fly *Drosophila melanogaster* (Butler, 1961). Moline (1984a) compared the aggressiveness of two spices of *Geotrichum* viz., *G. penicillarum* and *G. candidum* and found the latter to be more pathogenic and aggressive.

Transit losses in packed tomatoes was reported as a serious problem by Eckert and Kolbezen (1962). *Rhizopus nigricans* was found to be associated and produced characteristic nests of rotted fruits.

The symptoms due to *Fusarium* sp. started as water soaked lesions. This later increased in size. The tissue beneath got depressed , sunken and irregular cracks appeared occasionally. Tissues adjoining the lesions became soft with a cottony growth is seen (Thakur and Yadav, 1971).

Mucor sp. is recorded to attack red and ripened tomatoes. The fruit shows a watery area which is soft to touch. It is accompanied by a watery ooze. When the whole fruit rots, the growth of *Mucor* becomes visible (Moline, 1984b).

Okoli and Erinle (1990) studied the rate of rot induced on stored tomato fruits and found *Fusarium equisetti, F. oxysporum, F. chlamydosporum, Rhizopus stolonifer, Collelotrichum capsici, Geotrichum candidum* and *Curvularia lunata* as causal agents. *R. stolonifer* was the fastest in inducing rot while *C. lunata* was the slowest.

Of the six new post harvest fungal diseases reported by Shrivastava *et al.* (1993) the fruit rot caused by *Penicillium aurantiogriseum* on tomatoes is the most pathogenic one among them.

2.2 Epiphytic microflora of fruits and their isolation

It is well recognized that leaf surface and fruit surface constitute a distinct microhabitat which is inhabited by a varied assemblage of saprophytic and parasitic microorganisms. They include fungi, bacteria, actinomycetes, and yeasts. Much work on the microflora of the fruit surface has been done outside India.

Beneke *et al.* (1954) isolated fruit surface microflora of strawberry by placing cut plugs of host tissue on solid media and obtained fungi like *Rhizopus stolonifer*, *Cladosporium* sp., *Alternaria* sp., *Aspergillus* sp. and *Trichoderma* sp.

Dennis and Mountford (1975) analysed the fungal flora of freshly harvested raspberry fruit washings and obtained *Sporotrichum* sp., *Humicola* sp., *Phoma* sp., *Cryptococcus* sp. and *Torulopsis bacarum* as the dominant flora.

Dennis (1976) assessed the microflora of soft fruits in UK by macerating the whole fruit in a blender. He obtained *Geotrichum candidum*, *Epicoccum purpurascens*, *Phoma* sp., *Sporobolomyces roseus*, *Cryptococcus diffidenie*, *Acremonium* sp. etc. However this method was not suitable for distinguishing the surface dwellers and internal microorganisms.

Tronsmo and Dennis (1977) using the fruit washings of fresh strawberries obtained *Penicillum* sp., *Cladosporium* sp., *Aureobasidium pullulans* and many types of yeasts and surface microorganisms. In a similar study with grapes experiment Bisiach *et al.* (1985) obtained four strains of *Trichoderma viride*, five strains of *Trichoderma harzianum*, one strain of *T. hamatum*, *Aureobasidium* sp and *Cladosporium* sp.

Madhukar and Reddy (1987) have also used fruit washings as a method to obtain the epiphytic microflora of Guava at Warangal, Andhra Pradesh and reported the occurrence of *Aspergillus nidulans, C. cladosporiodes, Pestalotiopsis versicolour, R. stolonifer, Penicillium oxalicum, Fusarium moniliformae, A. terreus* and *A. niger* as the predominant ones along with several other fungi.

Sen (1989) tried stamping of leaves, leaf washings and blending as a method to obtain epiphytic microflora and observed the blending method to have yielded the maximum number of colony forming units (c.f.u.).

Chalutz and Wilson (1990) obtained bacteria and yeast from the diluted washings of citrus fruit.

McLaughlin *et al.* (1992) succeeded in isolating *Candida guillermondii* from the surface of fresh lemon and found it to be effective in the control of rots caused by *Monilinia fructicola, Rhizopus stolonifer, Botrytis cinerea* and *Penicillium expansum* on lemon fruits.

Wolfgang *et al.* (1997) isolated *Aureobasidium pullulans, Rhodotorula glutinis* and *Bacillus subtilis* from the surface of untreated apples in South West Germany and found it to be effective in reducing the decay caused by *Pencillium expansum, Pezicula malicorlius* and *Botrytis cinerea.*

The peels and wounded areas when shaken in phosphate buffer yielded the yeast *Candida sake* and this was used as a post harvest application to control blue mold of apples (Texido *et al.*, 1998).

2.3 Studies on interaction between major pathogens, epiphytic microorganisms and their mode of antagonism

The interaction between pathogen and microflora along with their mode of antagonism has been well documented.

Boosalis in 1964 observed parasitism and destruction of the conidia of *Helminthosporium sativum* by *Gliocladium roseum*.

Tronsmo and Dennis (1977) recorded the control *Botrytis cinerea* by both direct parasitism and antibiotic production under *in vitro* conditions by *Trichoderma* sp

Singh *et al.* (1978) have also recorded similar results with *Acremonium sordidulum* and found it to be mycoparasitic on *Collelotrichum dematium f. truncata* .

The biochemical nature of antagonism was also studied. Thus Hadar *et al.*(1979) have reported the production of extracellular enzymes in the region of interaction by *T. harzianum* upon *R. solani*.

Arora and Dwivedi (1980) observed the inhibition of *R. solani* through hyphal coiling and penetration by *Fusarium* sp.

Elad *et al.* (1980) have confirmed the above result and observed a similar mode of antagonism against *Sclerotium rolfsii* by *T. harzianum*.

Behamou and Chet (1983) studied the interaction between *Trichoderma harzianum* and *R. solani* in detail and concluded that *R. solani* was inhibited by

chitinase produced by *T. harzianum* leading to osmotic imbalances and intracellular disorders on plasma membrane.

Gupta *et al.* (1983) recorded destruction of *Rhizopus stolonifer* by coiling, penetration and coagulation of the hyphal contents by *Dreshlera rostrata*.

Production of enzymes were also reported, thus *Trichoderma harzianum* isolate 203 attacked the sclerotia of *S. rolfsii* with production of chitinase and glucanase enzymes (Elad *et al.*, 1984).

Lifshitz *et al.* (1984) have stated that *Pythium nunn* inhibited the growth of *P. vexans* and *P. ultimum* by coiling and *R. solani* through the formation of appresoria like structures.

Similarly while examining biological control of *Macrophomina phaseolina* by *T. harzianum* in dual hybrid culture, Elad *et al.* (1986) observed inhibition of microsclerotia formation.

Parasitism of *T. viride* and *Penicillium oxalicum* on *R. solani* by coiling and penetration is observed by Gokulapalan (1989).

Similarly Padmakumari (1989) found *Aspergillus niger*, *Chetomium globosum*, *Fusarium semitectum*, *F. solani*, *Gliocladium virens*, *Neurospora crassa*, *T. viride* and *T. harzianum* to be antagonistic to *R. solani* under *in vitro* conditions.

Casida and Lukezic (1992) while attempting to control leaf spot diseases of tomato and alfalfa using *Pseudomonas* strain 6792 observed the production of antifungal compounds that inhibited *Alternaria solani* and *Phoma indicaginis*.

Reeny (1995) in her studies with *Trichoderma viride* found that it controlled *Fusarium solani* by coiling and penetration and *R. solani* by coiling and disintegration. But only penetration of hyphal cells was noticed with *Colletotrichum capsici*.

Inbar *et al.*, 1996 recorded coiling and disintegration by *T. harzianum* on *Sclerotinia sclerotiorum*.

2.4 Mass multiplication and methods of application of the antagonists

Different types of materials have been tried for the mass multiplication of antagonists. The mass multiplied antagonists were formulated and applied in different ways.

Hunt *et al.* (1971) formulated *Trichoderma* sp. in chain saw oil (motor oil) and successfully inoculated it on pine stumps during tree cutting to control root rot pathogens in forest trees.

Homez *et al.* (1972) attempted mass multiplication of *T. harzianum* by seeding an autoclaved mixture of 1g ground annual rye grass seed, 10 g sandy loam and 2 ml water after shaking for 10 days.

Blackman and Rodriguez - Kabana (1975) obtained good results with *T. harzianum* when diatomaceous earth granules were impregnated with 10 per cent molasses solution.

Sterilized coniferous bark compressed to 1 cm pellets is also used as a method to proliferate *Trichoderma viride*. The commercial preparation BINAB - T is of this type (Gindart and Ricard, 1976).

Kelly (1976) evaluated the viability of *T. harzianum* and found its viability to be at satisfactory levels even after four months.

A mixture of wheat bran and tap water (1 : 2 v / v) was used as medium for multiplication and dispersal with satisfactory results for *Trichoderma* by Hader *et al.* (1979).

Jones *et al.* (1984) while testing a carrier system, for being applied to soil, found ground lignite granules of size 425 to 2000 µm and twice amended with thin liquid stillage (byproduct of ethanol production) to be best for *T. harzianum* and *G. virens* with more than 90 per cent viability after four months.

A mixture of wheat bran and peat (1 : 1 v / v) was also found to be ideal for the mass culturing of *T. harzianum* in the control of damping off caused by *Pythium aphanidermatum* (Sivan *et al.*, 1984).

Lewis and Papavizas (1984) found bran : sand and water (1 : 1 : 2 w / w/ v) inoculated with conidia and incubated for 1 - 3 days to be best for the growth and proliferation of *Trichoderma* sp.

Spores of *T. hamatum*, *T. harzianum* and *T. viride* were found to tolerate metham in solution up to 350 µg a.i. / ml and hence have suggested the possibility of it being included in such formulations (Lewis and Papavizas, 1984).

Papavizas *et al.* (1984) used a broth of molasses and yeast to produce large batches of biomass of *Gliocladium virens*, *T. hamatum*, *T. harzianum* and *Talaromyces flavus*. After 15 days of growth, they found the mycelial mats contained 75 per cent chlamydospores. These mats were ground by them into 25

per cent or 50 per cent formulation in talc. Its storage life was best at -5^{0}C and 5^{0}C while least at 25^{0}C and 30^{0}C.

Boyette and Walker (1985) explored the possibility of containing soyabean pathogen by granulating mycelial inoculum of *Cercospora kikuchiana* in sodium alginate, kaolin clay into 0.25 m $CaCl_2$ solution and granules applied after air drying.

Frevel *et al.* (1985) tried the encapsulation of biocontrol agents in an aqueous solution containing one per cent sodium alginate and 10 per cent Pyrax R (anhydrous aluminium silicate), which was comminuted in a blender to which conidia of *Gliocladium* and *Trichoderma* were added and dried. This preparation retained its viability for four weeks.

Lewis and Papavizas (1985) studied the characteristics of alginate pellets formulated with *Trichoderma* and *Gliocladium*. They studied the pelletized formulation of wheat bran, or kaolin clay in an alginate gel containing conidia, chlamydospores or fermentation biomass of *Trichoderma* and *Gliocladium*.

Lutchmeah and Cooke (1985) studied pelleting of seed with the antagonist *Pythium oligandrum*. Oospores of *P. oligandrum* incorporated in the clay carrier, survived the commercial seed pelleting and gave protection against damping off in sugar beet.

Papavizas (1985) evaluated the ability of certain cations like Ca^{++} to form gels with aqueous solutions of sodium alginate that could improve delivery technology for *Trichoderma* and *Gliocladium*.

Strashnov *et al.* (1985) observed a reduction in the rotting of tomato fruits on coating with *T. harzianum* by 85 per cent under laboratory conditions and by 27-51 per cent under field conditions.

Pusey *et al.* (1988) conducted the pilot test for commercial production and application of *Bacillus subtilis* for post harvest control of peach brown rot. They incorporated the antagonists into wax on the package line and effectively controlled the brown rot incidence.

McLaughlin *et al.* (1992) successfully utilised the two yeasts *Kloeckera apiculata* and *Candida guilliermondii* against the diseases of grape, peach and apple.

Peng *et al.* (1992) tried a powder formulation of talc and cornmeal containing *Gliocladium roseum* at 5 x 10^8 c.f.u./g. Honeybees were used as vectors. The formulation was placed in dispensers inside bee hives. The bees crawled and carried the formulation on their legs in the process of pollination and effectively suppressed the infection by *Botrytis cinerea* on strawberry fruits in the field.

Sutton and Peng in 1993 observed that a concentration of 10^7 - 10^8 spores or cells / ml water as the optimum quantity to be applied on fruits as dip, spray or droplets.

Bacillus licheniformis as a post harvest dip application controlled anthracnose and stem end rot of mangoes in packing house (Korsten *et al.*, 1993).

Kammath (1994) mass multiplied the *Fusarium* species *viz. F. semitectum* and *F. solani* which was found to be pathogenic to the water hyacinth. The mass

multiplication was done on different media and found rice bran and wheat bran to be ideal for the mass multiplication.

Laurie *et al.* (1995) observed prestorage dip with the yeast suspension of *Candida oleophila*. This reduced the disease of nectarines caused by *Penicillium expansum*.

Prasad *et al.* (1997) tried the biomass production of *T. harzianum* in Potato Dextrose broth and compared it with V-8 juice broth and Molasses and Brewers yeast broth and found that Potato Dextrose broth gave higher quantity of biomass. Its storage was tested in eight type of formulations. It was found that in wheat bran and pesta granules, the number of viable propagules increased upto 30 days and then declined at room temperature. The number of propagules in other formulation decreased with time. The fungus survived through out the study (90 days) when stored at 4^0C, though propagules were least at the end of 90 day.

Sutton *et al.* (1997) studied *G. roseum* as a biological control agent effective against *Botrytis cinerea* on strawberry, geranium, tomato, black spruce and cucumber. The fungus easily produced abundant spores and the spores remained viable for a long time. The formulation was not harmful to humans or bees and posed no ecological risks.

Gracia - Graza (1998) used a formulation of *Fusarium oxysporum* f. sp. *erythroxylis* with wheat flour amended with olive oil. This was easily dispersed by ants and controlled the *Fusarium* wilt of Coca (*Erythroxylum coca*).

Lewis *et al.* (1998) Used formulation of *Trichoderma* and *Gliocladium* and observed its efficiency against damping off caused by *R. solani*. He used a commercial preparation of cellulose granules (Biodac) with a sticker.

Microbial antagonists are formulated as dry as well as wet types. The dry types includes dust, granules and briquettes, while the wet types are emulsions of water based solutions. Burges (1998) observed 18 months viability as a desirable character of a formulation.

Fravel *et al.* (1998) have listed about 40 products commercially available world over. Most of these are based on *Trichoderma* spp. Other commercial formulations available include those of *Fusarium oxysporum*, *Gliocladium* sp., *Candida oleofila* etc.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Isolation and identification of fungi

Diseased samples of brinjal, chilli and tomato were collected from the local markets of Thiruvananthapuram during the year 1997-98 at monthly intervals. The samples were collected separately in polyethene bags, brought to the laboratory and isolations were made within 24 hours of collection, care was taken so as to avoid over rotted vegetables.

Diseased samples were cut into small bits and surface sterilized by dipping in 0.1 per cent aqueous mercuric chloride for two minutes followed by washing in three changes of sterile distilled water. These bits were then transferred to sterilized and melted potato dextrose agar medium in petriplates (Appendix - I) and incubated at room temperature (28 ± 1^0C) for seven days. The fungal growth was transferred to the potato dextrose agar slants after purification by single spore isolation method. These culture were maintained by subculturing at monthly intervals and stored under refrigeration. The morphological and cultural characteristics were studied and cultures identified by slide culture technique of Riddel (1974).

Plain agar was melted and poured into petridishes to a thickness of 2 mm and after solidification blocks of 6 mm were cutout using a sterile needle. One such block was placed in the centre of a sterile microscopic slide and all the four sides of the agar block was inoculated with small bits of the fungus culture. A cover slip was placed on the top of the agar block and the slide was kept in a moist chamber and incubated for 48 hours at room temperature. The

cover slip was then gently lifted, a drop of 15 per cent alcohol was placed in the centre and before drying, the cover slip was mounted on lactophenol cotton blue. The cultures were examined and identified.

The pathogenicity of the isolates was proved following Koch's postulates. Fresh and healthy samples of brinjal, chilli and tomato were collected from local markets of Thiruvananthapuram. The vegetables were surface sterilized as detailed earlier and inoculations with different pathogens were carried out using the technique of Granger and Horne (1924). In this method a hole of 8 mm diameter was made on the surface of the fruit using a sterile cork borer. A fungal bit from an actively growing culture was kept in the hole and plugged with the cut piece and incubated at room temperature. Three replications were maintained with five fruits in each replication. The isolates that proved their pathogenicity were taken up for further studies.

The pathogens that occurred most frequently, were taken up for studies on interaction. These included :

Brinjal : *Phomopsis vexans*

Chilli : *Colletotrichum capsici*

Tomato : *Geotrichum candidum*, *Rhizoctonia solani*, *Fusarium solani*

3.2 Collection, isolation and identification of epiphytic microflora of healthy fruits

3.2.1 Collection of samples

Healthy and matured fruits of brinjal, chilli and tomato were collected from different markets of Thiruvananthapuram at five day interval for 45 days.

The fruits were individually packed in polyethene bags and brought to the laboratory for isolation studies.

3.2.2 Isolation and identification of epiphytic microorganisms

The epiphytic microflora on the fruit surface was examined using dilution plate technique (Timonin, 1940) with slight modifications using Peptone Dextrose Agar with Rose Bengal (Appendix - II).

A medium sized brinjal fruit of 6 cm diameter and 10 - 15 cm length was used. The peel of the fruit was removed using a blade which had been dipped in 95 per cent alcohol and flamed. One gram of the peel was transferred to 99 ml of sterile distilled water in a 250 ml conical flask and shaken for 20 minutes in a rotary shaker. From this the final dilution of 10^4 was prepared.

For chilli and tomato the same procedure of the dilution with the whole fruit was adopted. A dilution of 10^3 was used. From the final dilution one ml of the solution was mixed with 9 ml of Peptone Dextrose Agar with Rose Bengal medium kept at 45^0C and poured into petriplates and rotated to ensure uniform spread of the suspension in the media. Three replications were maintained for each fruit. The dishes were incubated at room temperature (28 $\pm$ 1^0C). Immediately after the development of the colonies, they were subcultured in PDA slants and subsequently purified by the hyphal tip culture method. The purified cultures were then stored under refrigerated conditions for further studies. The fungi were then identified using standard techniques as suggested by Riddel (1974).

3.3 Studies on mycoparasitism

The dual culture technique outlined by Dickinson and Skidmore (1976) was followed for studying the antagonistic activity. Discs of 5 mm diameter taken from the edge of vigourously growing colonies of the selected pathogen and the epiphytic fungi were placed 3.5 cm apart on PDA in petridishes and incubated at room temperature for ten days. Control consisted of petridishes inoculated with culture discs of 5 mm diameter of the selected pathogens. The plates were examined at 24 hour interval and growth recorded.

After the fungus growth in the control plates has completely covered the petridishes, observation on the interaction and its classification were made using the method of Purkayastha and Bhattacharya (1982) with five groups as follows :

A. Homogenous - Intermingling between organisms

B. Overgrowth - Pathogen overgrown by test fungus

C. Cessation of growth at the line of contact

D. Clear zone of inhibition

E. Overgrowth : Test fungus overgrown by pathogens

All the isolated epiphytes were tested and the most promising were found to be *Trichoderma harzianum* and *Gliocladium virens*. These two fungi were used for further study.The culture of *T. viride* obtained during the studies conducted earlier in the department of plant pathology, College of Agriculture, Vellayani (Reeny 1995) was also included as a check.

The data obtained was statistically analyzed using the method outlined by Panse and Sukhatme (1954).

3.4 Mechanism of mycoparasitism

The dual culture technique of Dennis and Webster (1971) was used for studying the mechanism of antagonism. In sterile petridishes melted PDA was poured and allowed to solidify. Sterilized cellophane discs of 90 mm diameter were placed over this so as to lie flat on the medium, using a pair of sterile forceps. An agar disc of 5 mm diameter containing the mycelium cut from the margin of an actively growing culture of the fungal pathogen was placed 2 cm apart along with a 5 mm agar disc of the test fungus. The plates were incubated at 28 ± 1^0C for 48 hours. Direct observations were carried out after incubation period under a light microscope at the zone of hyphal contact. Observation for hyphal interaction was also made by cutting out one square centimeter portions of cellophane containing intermingling hyphal growth and mounted on glycerin. The different mechanisms of mycoparasitism exhibited by the efficient antagonists of fruit rot pathogens were also studied.

3.5 *In vivo* testing of the antagonistic microorganisms

The water suspension of the spores of antagonists viz., *Gliocladium virens* and *Trichoderma harzianum* was prepared by mixing the spores in sterile distilled water. The spore load of the antagonist was maintained at 10^8 / ml. The spore count was made using a haemocytometer. One gram of Carboxy Methyl Cellulose (CMC) was added to the spore suspension to increase the adhesiveness of the spores. This spore suspension was used to treat the fruits. The fruits were dipped for two minutes in the spore suspension and air dried at room temperature. The fruits sprayed with distilled

water served as the control. To study the effect of antagonists on the pathogenic fungi, the fruits were surface sterilized, injured by pin-pricks and inoculated with the seven day old culture of the pathogen. All the treatments were replicated thrice and each replication consisted of five fruits. The number of fruits infected in each replication was counted and expressed as per cent infection. The observations were made for eight days.

3.6 Effect of carrier materials on the viability and shelf life of the fungal antagonist (*T. harzianum*)

Four carrier materials were tested. This included :

1) Coir pith

2) Wheat Flour

3) Charcoal

4) Talc

Raw wheat flour (unseived) obtained from the market was tied in a muslin cloth, autoclaved for 20 minutes and cooled. To 25 g of this, kept in a conical flask, 10 ml sterile water was added. Coir pith, talc, charcoal (powdered) were also autoclaved and wetted in a similar way. To each of the above flasks 10 ml of a spore suspension obtained from a profusely sporulating culture was added asceptically and stored at room temperature.

To study the effect of carrier material on the viability of the spores the following procedure was adopted. One hundred mg of these materials was taken in a test tube and shaken with five ml sterile water at weekly intervals to release all spores into suspension.

One drop of the above suspension was kept in a cavity slide. To this one drop of 40 per cent glucose solution was added and the average number of spores per field were counted. The germination percentage of the spores was noted after 24 hours. Based on the observations talc was used for further studies.

3.6.1 Formulation of the antagonist in talc

3.6.1.1 Production of the mycelial mat

For the production of mycelial mat the liquid fermentation technology of (Papavizas *et al.*, 1984) with slight alterations was used. One hundred ml of a broth containing 30 per cent of jaggery and five per cent brewer yeast in 250 ml conical flask was autoclaved at for 20 minutes and cooled. This was then inoculated with a culture disc of 1 cm in diameter of the antagonists and incubated at room temperature for 15 days to obtain sufficient propagules in the mycelial mat. The mat was then removed from the conical flask and dried for three days. This mat was used for further study.

3.6.1.2 Grinding and preparation of formulation

The air dried mycelial mat was ground and mixed (25 per cent w/w) with talc. The powdered material obtained thus was stored under refrigerated condition.

3.6.1.3 Viability and survival of propagules in talc based formulation

Hundred milligram of the formulation was mixed with 100 ml sterile water and mixed thoroughly by shaking in a shaker for ten minutes. One ml of this suspension was taken out with a sterile pipette and transferred to a conical flask containing 99 ml sterile water. From this 1 ml was transferred to 9 ml of sterile water and the process was repeated till 10^5, 10^6 and 10^7 dilutions were obtained. One ml of the aliquot from each of these dilutions were taken asceptically and placed in a sterile petridish.

Martins Rose Bengal medium (Appendix - II) was melted and cooled and 15 ml of the media was poured into the above petridishes. The petridishes were shaken by swirling motion of the hand and incubated at room temperature.

The colonies were counted on the fourth day and such observations were taken upto six weeks at weekly intervals and the number of viable colony forming units (c.f.u.) per gram of the formulation was also calculated.

3.7 Comparative study of the different methods of application of the antagonist

3.7.1 Effect of dipping

For studying the effect of dipping five per cent solution of starch (general reagent) mixed with five gram of the formulation (powdered dry matter of antagonist, *T. harzianum* + talc) in 1 : 3 proportion was used. The extent of rotting was recorded after specific time intervals.

3.7.2 Effect of dusting

For studying the effect of dusting, five grams of the formulation (powdered dry matter of antagonist, *T. harzianum* + talc) in 1 : 3 proportion was used.

Samples of chilli, brinjal and tomato washed, dried and inoculated with their respective pathogens were used for the study. The treatments included :

T_1 = Control (inoculated with the pathogen)

T_2 = T_1 followed by dipping with the antagonistic formulation

T_3 = T_1 followed by dusting with the antagonistic formulation

Three replications were maintained for each treatment. Each replication consisted of ten numbers of chilli and five numbers of brinjal and tomato. The observations were taken for eight days at four day interval.

RESULTS

4. RESULTS

4.1 Isolation of fungi associated with the spoilage of solanaceous vegetables and their identification

The following fungi were found associated with spoilage of three solanaceous vegetables viz., brinjal, chilli and tomato.

4.1.1 Brinjal : *Solanum melongena* L.

Phomopsis vexans (Sacc. and Syd.) Harter

Phytophthora capsici Leonian

Pythium aphanidermatum (Eds.) Fitz.

Aspergillus terreus Thom.

The pathogenicity tests revealed the following :

Fungi	Pathogenicity	Days taken for infection
P. vexans	+	2
P. capsici	+	3
P. aphanidermatum	+	3
A. terreus	-	-

P. vexans and found to be associated most frequently and hence was selected for further study.

4.1.1.1 Cultural characteristics and symptoms produced

P. vexans

Initially the growth was submerged and white in colour. On aging it turned brownish. The pycinidia was observed after 20 days of growth and

measured 0.5 to 1.5 mm in diameter. Pycniospores were hyaline and one celled and measured 1 - 1.3 x 1.9-2.3 µm. Stylospores were filiform, curved, hyaline and aseptate and measured 0.72 - 1.2 x 3.5 - 4.2 µm. The colonies attained 9 cm growth in 8 days (Plate 1).

Based on the above cultural and conidial characteristics the fungus was identified as *Phomopsis vexans.*

On the fruits the infection started as light brown, slightly depressed more or less circular spots. Gradually this enlarged and by four days attained a diameter of four centimeters. The infected area was clearly demarcated from the healthy tissues. Extensive rotting of the pulp was also noticed. The skin became soft, got wrinkled and shrunken (Plate 2).

4.1.2 Chilli : *Capsicum annuum* L.

Colletotrichum capsici (H. Syd.) E Butler and Bisby

Phytophthora capsici Leonian

Alternaria alternata (Fr.) Keissl

Botrytis cinerea Pers.

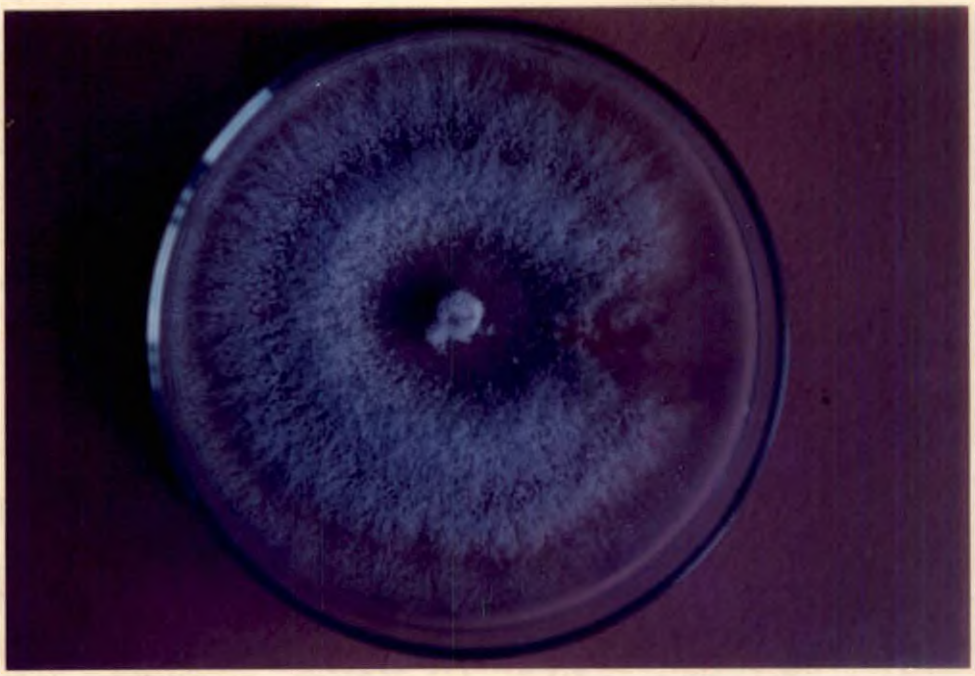
Aspergillus terreus Thom.

The pathogenicity tests revealed the following.

Fungi	Pathogenicity	Days taken for infection
1. *C. capsici*	+	2
2. *P. capsici*	+	2
3. *A. alternata*	+	3
4. *B. cinerea*	+	4
5. *A. terreus*	-	-

Plate 1 Growth of *Phomopsis vexans* on PDA seven days after inoculation

Plate 2 Symptom of *P. vexans* on brinjal fruits eight days after inoculation



Colletotrichum capsici was frequently found to be associated with fruit rot and hence selected for further studies.

4.1.2.1 Cultural characteristics and symptoms produced

C. capsici

Colonies were white on PDA. On the reverse side, the colour was slightly brown. Setae abundant and conidia formed in pale buff masses, fusiform and generally tapering to the end, with 20.4 x 3.1 µm dimensions. Colonies attained 9 cm growth in diameter by 8 days on PDA at 28 ± 1^{0}C with very sparse aerial hyphae (Plate 3).

Based on the conidial morphology and cultural characteristics the fungi was identified as *C. capsici*.

On the fruits the symptoms appeared as small, water soaked circular and sunken spot. Within four days the centre turned straw coloured and later became blackish brown. Numerous acervuli appeared as black pustules in concentric rings. Black setae were also noticed arising from pycnidia (Plate 4).

4.1.3 Tomato : *Lycopersicon esculentum* Mill.

Geotrichum candidum Link. ex Leman

Rhizoctonia solani. Kuhn.

Fusarium solani (Mart.) Sacc.

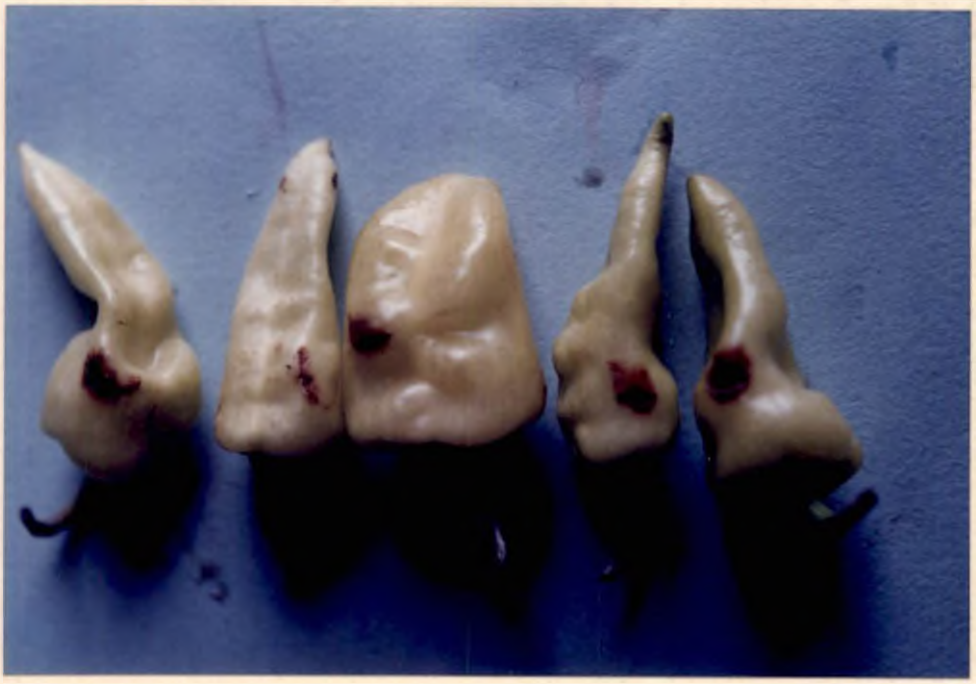
Rhizopus stolonifer (Ehrab ex Fr.) Lind

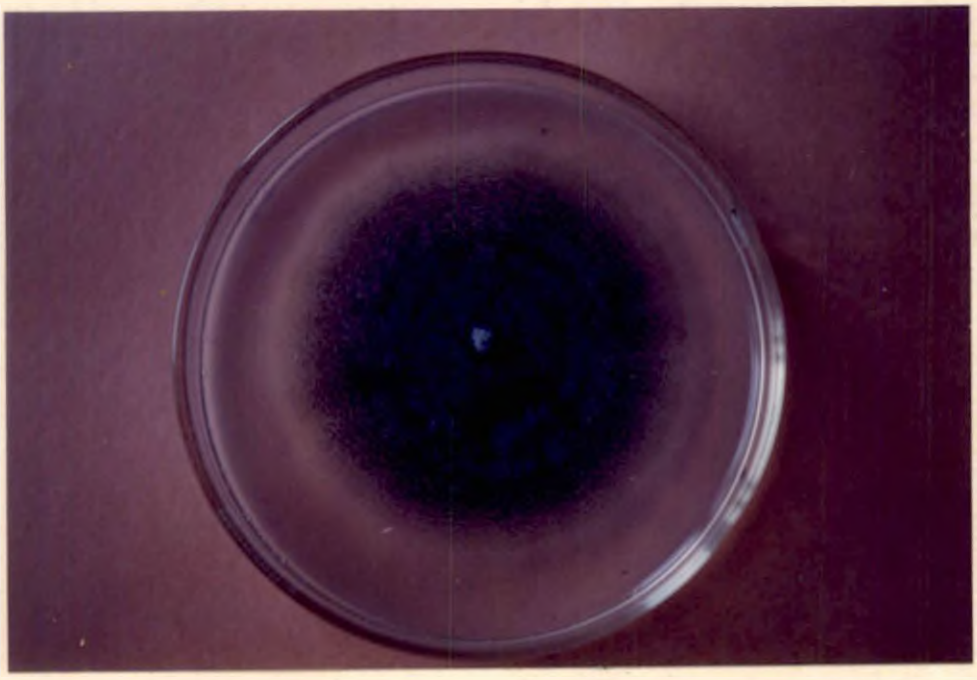
Pythium aphanidermatum (Eds.) Fitz.

Alternaria alternata (Fr.) Keissel.

Plate 3 Growth of *Colletotrichum capsici* on PDA seven days after inoculation

Plate 4 Symptom of *C. capsici* on chilli fruits eight days after inoculation





The pathogenicity tests revealed the following.

Fungi	Pathogenicity	Days taken for infection
A. alternata	+	3
F. solani	+	1
G. candidum	+	2
P. aphanidermatum	+	2
R. solani	+	2
R. stolonifer	+	1

G. candidum, *R. solani* and *F. solani* were isolated frequently and hence were selected for further studies.

4.1.3.1 Cultural characteristics and symptoms produced

Geotrichum candidum

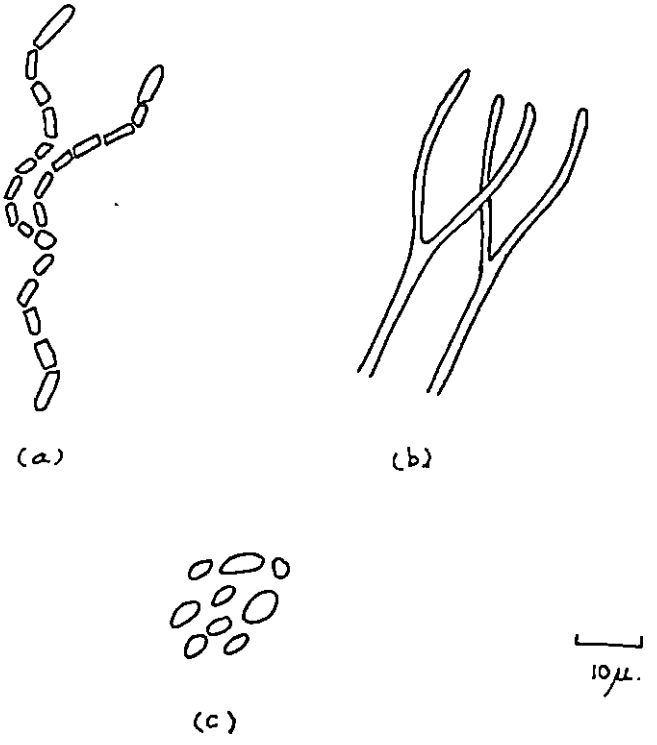
The colonies were white powdery in appearance with distinct fruity odour, submerged septate hyphae and reached 9 cm in 7 days on PDA at 28 ± 1^0 C. The advancing hyphae were dichotomously branched. The average diameter of the hyphae was found to be 6.3 µm. Conidia is formed as arthroconidia in chains measured 6-12 x 3.6 µm (Plate 5, Fig. 1).

Based on the above cultural characteristics the fungi was identified as *G. candidum*.

On the fruits, the symptom initiated as a small rotted area which was soft to touch. By second day a wide 'star' shaped crack appeared and rotting was initiated. The white scum like growth of the pathogen along the crack was

Fig. 1 Morphology of *Geotrichum candidum*

(a) Arthroconidia
(b) Dichotomous hyphal tip
(c) Mature conidia


(a)
(b)
(c)
10μ.

visible. The rotted fruit gave a sour and sweet fruity odour. By 5 days the fruit was slightly shriveled with exudation and showed profuse white growth on the fruit (Plate 6).

Fusarium solani

The colonies were fast growing and reached 9 cm diameter in 7 days on PDA at 28 ± 1^0 C. The mycelia was light brown in colour. Micro conidia were sparse and were produced on conidiophores. Microconidia measured 8-16 x 2-4 µm. Microconidia were present in abundance. The macroconidia is moderately curved with 2 - 3 septa and measured 20 - 23 x 2 - 4 µm. Chlamydospores were sparse, occurred singly or in pairs in chains and measured 6.3 - 7.2 µm in diameter. The PDA was imparted a buff colour (Plate 7).

Based on the conidial morphology and cultural characteristics the fungi was identified as *F. solani*.

On the fruits, infection started as water soaked lesions which were initially raised but later became depressed. Irregular cracking of fruits was seen. Dirty white mycelia was seen along the cracks. The disease progressed rapidly, disintegrated the inner tissue which lost turgidity and resulted in exudation of yellow juice with unpleasant odour (Plate 8).

Rhizoctonia solani

The colony was white initially and later turned brown and completely covered the petridish (9 cm) in 6 days on PDA at 28 ± 1^0 C. The sclerotia were present in abundance. Sclerotia are irregular in outline, often solitary about

Plate 5 Growth of *Geotrichum candidum* on PDA seven days after inoculation

Plate 6 Symptom of *G. candidum* on tomato fruits eight days after inoculation

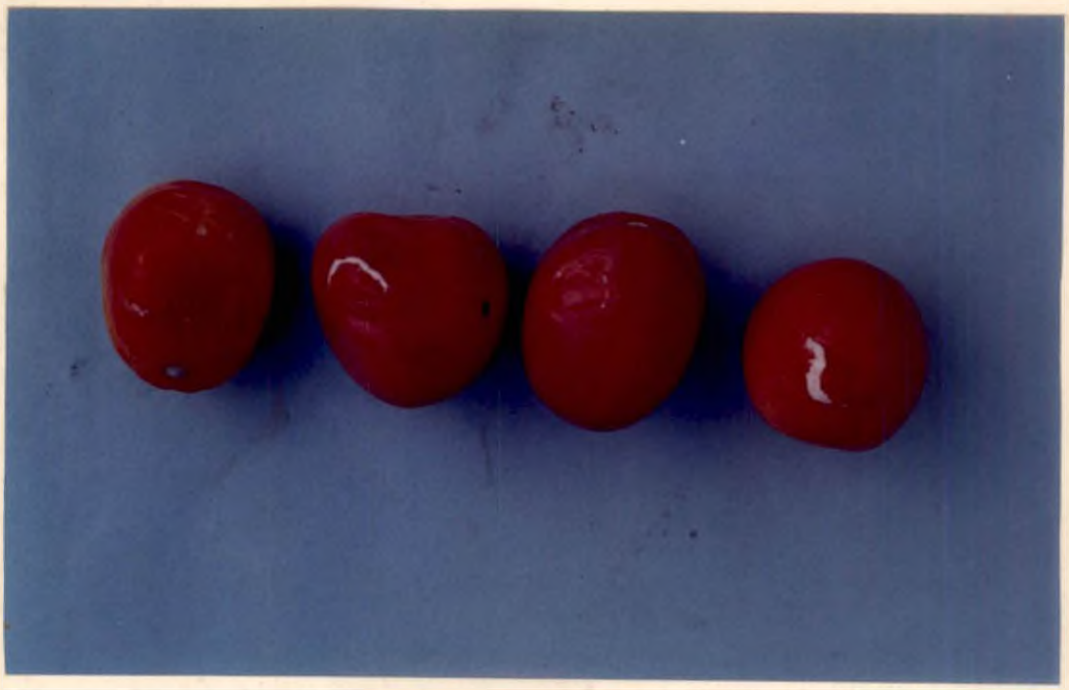
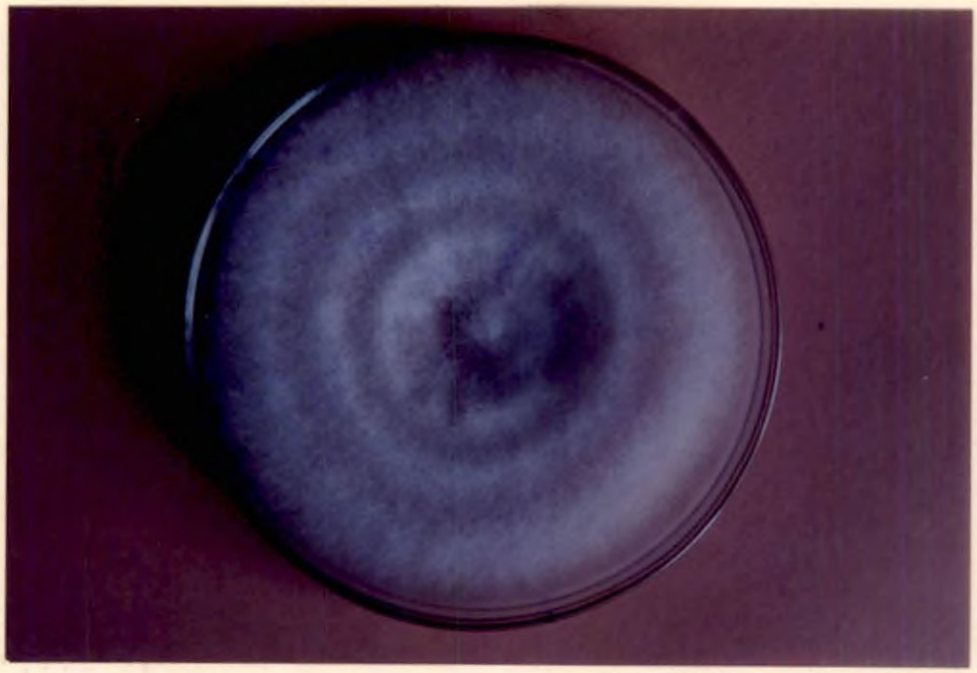
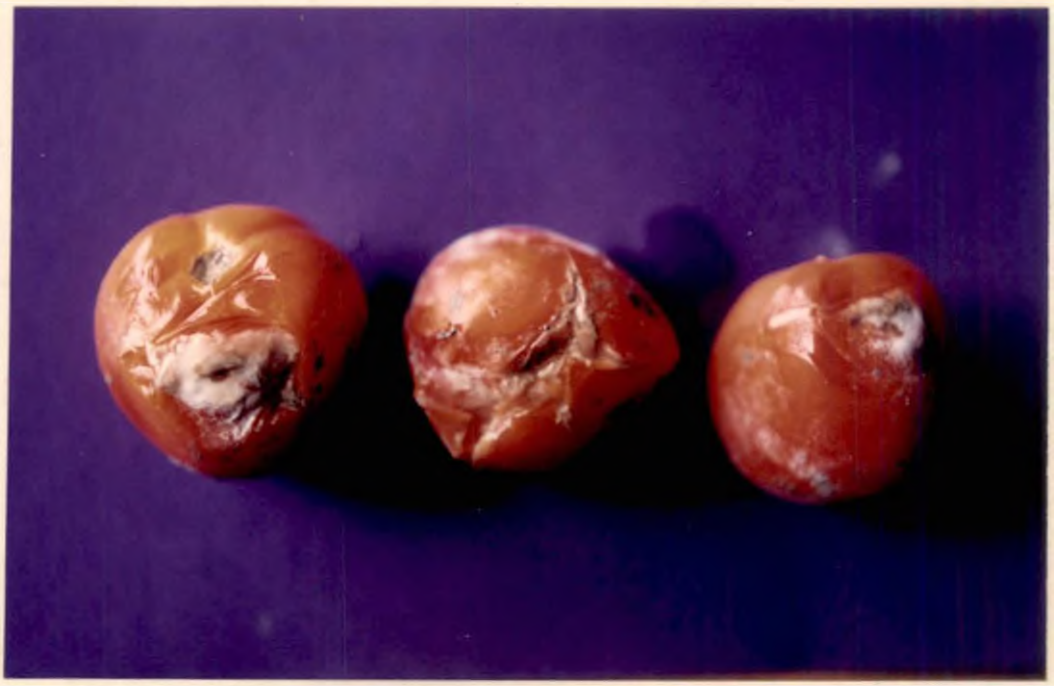


Plate 7 Growth of *Fusarium solani* on PDA seven days after inoculation

Plate 8 Symptom of *F. solani* on tomato fruits eight days after inoculation





1 mm in diameter, present often in zones particularly near the colony margin. They turn brown. The average diameter of hyphae was 8.3 µm. The lateral branches from the main hypha were constricted at the point of origin and septa occurred in the branches near the junction with the main axis (Plate 9).

Based on the cultural characteristics the fungi was identified as *R. solani.*

Lesions were roughly circular with a brown border and pale centre. As the infected area enlarged it became slightly sunken and remained firm. Splitting of the skin occurred in different directions. Inner tissues became soft and watery later (Plate 10).

4.2 Collection, isolation and identification of epiphytic mycoflora:

4.2.1 Brinjal : Following fungi were isolated from the matured and healthy brinjal fruit

1. *Aspergillus aculeatus* Iizuka
2. *A. ochraceous* Wilhem.
3. *A. terreus* Thom
4. *A. niger* van Teigh.
5. *Alternaria solani* (Fr.) Keissel
6. *Botryodiplodia theobromae* Pat
7. *Cryptococcus* sp Kutzing emend phaff et Spencer
8. *Fusarium solani* (Martius.) Sacc.
9. *Gliocladium virens* Miller, Gieldens and Foster
10. *Helminthosporium* sp.

Plate 9 Growth of *Rhizoctonia solani* on PDA seven days after inoculation

Plate 10 Symptom of *R. solani* on tomato fruits eight days after inoculation

11. *Penicillium wortmanii* Kloecker

12. *Penicillium notatum* Westling

13. *Pestalotiopsis palmarum* Cooke

14. *Phomopsis vexans* Sacc and Sydow

15. *Pythium aphanidermatum* (Eds.) Fitz.

16. *Rhizopus* sp. Ehrenb

17. *Sporobolomyces roseus* Kluyer et van Niel.

18. *Trichoderma harzianum* Rifai

4.2.2 Chilli : Following fungi were isolated from the surface of the healthy chilli fruits:

1. *Aspergillus flavus* Link ex. Fr.
2. *A. niger* van Teigh
3. *A. terreus* Thom
4. *A. aculeatus* Iizuka
5. *Cladosporium cladosporioides* (Fres.) de. Vries
6. *Cryptococcus* sp. Kutzing emend. Phaff et. Spencer
7. *Curvularia lunata* (Wakkar) Boedijn
8. *Fusarium solani* (Martius). Sacc.
9. *F. tricinctum* (Corda) Sacc.
10. *Penicillium oxalicum* Currie and Thom
11. *P. wortmanii* Kloecker
12. *Stigmina* sp.

4.2.3 Tomato : Following fungi were isolated from the surface of the healthy: tomato fruit :

1. *Aspergillus aculeatus* Iizuka
2. *A. flavus* Link ex Fr.
3. *A. niger* van Tiegh
4. *A. terreus* Thom
5. *Alternaria alternata* (Fr.) Keissel
6. *Cryptococcus* sp. Kutzing emend. Phaff et Spencer
7. *Fusarium solani* (Mart.) Sacc.
8. *F. oxysporum* Schlect
9. *Geotrichum candidum* Link ex Leman
10. *Penicillium notatum* Wesling
11. *P. wortmanii* Kloecker
12. *Pythium aphanidermatum* (Edz.) Fitz
13. *Sporobolomyces roseus* Kluyver et ran Miel
14. *Syncephalastrum* sp. Schrot

4.3 Studies on mycoparasitism

In vitro studies for selection of suitable mycoparasite :

4.3.1 Brinjal

The mean table showing the diameters of *Phomopsis vexans* and the epiphytic mycoflora of brinjal along with the colony interaction type is given below (six days after inoculation).

Table 1 Mean colony diameter of *P. vexans* and the interaction type with the epiphytes tested

Epiphytes tested	Mean colony diameter (cm)	Colony interaction type
A. aculeatus	6.0	C
A. flavus	6.8	C
A. niger	6.5	C
A. ochraceous	6.83	C
A. terreus	7.13	C
Alternaria alternata	5.5	C
Botryodiplodia theobromae	6.1	C
Cryptococcus sp	7.8	E
Fusarium solani	6.18	C
Gliocladium virens	1.5	B
Helminthosporium sp	3.0	C
P. wortmanii	5.76	D
Penicillium notatum	5.93	D
Pestalotiopsis palmarum	6.6	C
Pythium aphanidermatum	7.65	C
Rhizopus sp.	6.16	C
Sporobolomyces roseus	6.23	C
Trichoderma harzianum	2.0	B
C.D.	0.766	

B : Pathogen overgrown by test fungus; C : Cessation of growth at the line of contact;

D : Clear zone of inhibition ; E : Test fungus overgrown by pathogen.

All the epiphytes isolated were tested against *Phomopsis vexans*.

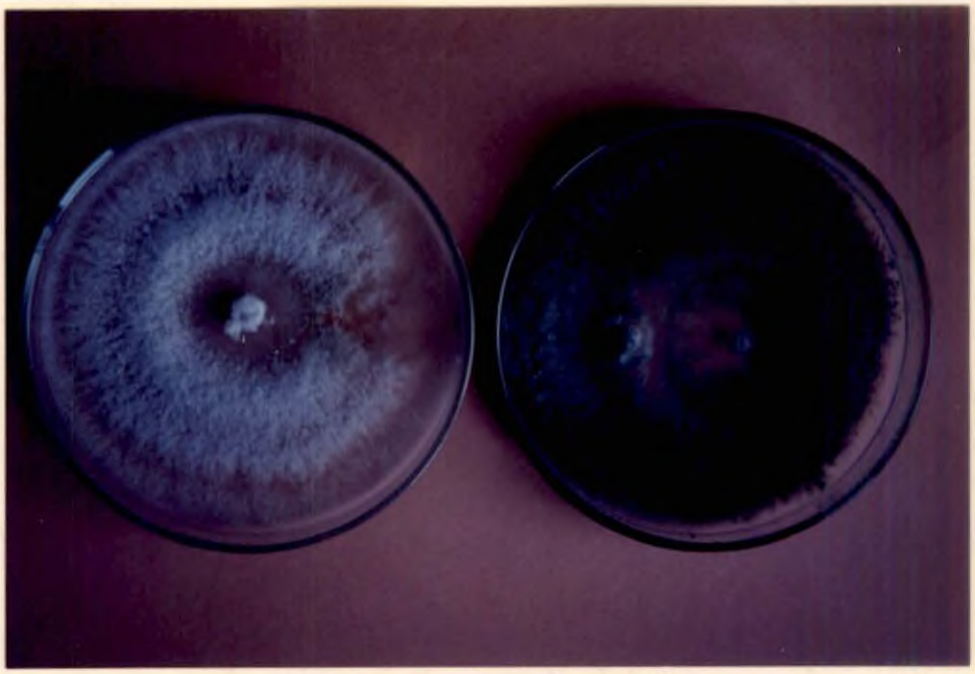
G. virens and *T. harzianum* showed over growth (B) (Plate 11, 12).

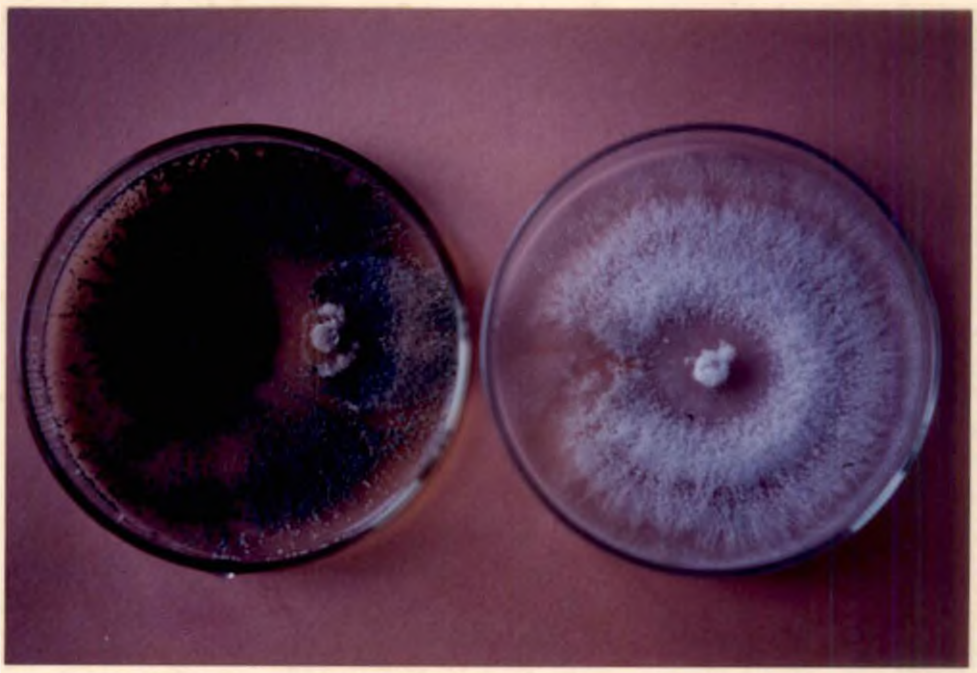
Plate 11 Colony interaction between *Gliocladium virens* and *P. vexans* showing overgrowth

Left : Control (*P. vexans*)
Right : *P. vexans* x *G. virens*

Plate 12 Colony interaction between *Trichoderma harzianum* and *P. vexans* showing overgrowth

Left : Control (*P. vexans*)
Right : *P. vexans* x *T. harzianum*





Aspergillus niger, *A. aculeatus*, *A. terreus*, *A. ochraceous*, *A. flavus*, *Botryodiplodia theobromae*, *Fusarium solani*, *Pythium aphanidermatum*, *Pestalotiopsis palmarum*, *Rhizopus* sp. and *Alternaria solani* showed cessation of growth at the point of contact (C). *Pencillium notatum* and *P. wortmanii* showed a clear zone of inhibition (D). *Cryptococcus* sp. was overgrown by the pathogen (E).

Since maximum inhibition of the pathogen (*P. vexans*) was shown by *G. virens* and *T. harzianum* and hence they were taken up for further study.

4.3.2 Chilli

The mean table showing the diameters of *Colletotrichum capsici* and the epiphytic mycoflora of chilli along with the colony interaction type is given below (seven days after inoculation).

Table 2 Mean colony diameter of *C. capsici* and the interaction type with the epiphytes tested

Epiphyte tested	Mean colony diameter (cm)	Colony interaction type
Aspergillus aculeatus	3.67	D
A. flavus	4.25	C
A. niger	3.65	B
A. terreus	3.23	B
Cladosporium cladosporioides	4.5	B
Cryptococcus sp.	4.5	C
Curvularia lunata	4.0	C
Fusarium tricinctum	5.23	C
F. solani	3.46	C
Pencillium oxalicum	4.26	C
Sporobolomyces roseus	6.1	C
Stigmina sp.	4.3	C
C.D.	0.456	

B : Pathogen overgrown by test fungus, C : Cessation of growth at the line of contact;
D : Clear zone of inhibition.

Twelve epiphytes were tested against the pathogen *C. capsici*. *Cladosporium cladosporiodes*, *Aspergillus niger* and *A. terreus* inhibited the pathogen by overgrowth (B). *Sporobolomyces roseus*, *Curvularia lunata*, *A. flavus*, *Stigmina* sp., *Penicillium oxalicum*, *Fusarium solani*, *Cryptococcus* sp. and *F. tricinctum* showed cessation of growth at the point of contact (C). *A. aculeatus* showed a clear zone of inhibition (D).

A. niger and *A. terreus* were found to be superior.

4.3.3 Tomato

The mean table showing the diameters of pathogens of tomato viz., *Geotrichum candidum*, *Fusarium solani* and *Rhizoctonia solani* and the epiphytic mycoflora of tomato along with the colony interaction type is given below (Table 3).

Table 3 Mean colony diameter of *Geotrichum candidum, Fusarium solani* and *Rhizoctonia solani* and the interaction type with the epiphytes tested

Epiphyte tested	*G. candidum*		*F. solani*		*R. solani*	
	Colony diameter (cm)	Interaction type	Colony diameter (cm)	Interaction type	Colony diameter (cm)	Interaction type
Aspergillus aculeatus	2.5	B	6.35	D	5.40	D
A. flavus	2.6	B	6.25	C	6.17	C
A. niger	2.7	B	6.33	C	5.93	C
A. terreus	2.2	B	4.75	D	5.00	D
Alternaria alternata	2.5	B	6.65	C	6.33	C
Cryptococcus sp.	5.0	C	7.0	E	9.00	E
Fusarium oxysporum	2.9	B	7.0	C	6.30	C
Fusarium solani	3.0	B	9.0	A	7.00	C
Penicillium wortmanii	3.0	D	6.0	D	6.83	D
P. notatum	2.5	D	6.2	D	6.30	D
Pythium aphanidermatum	3.0	B	6.5	C	7.00	C
Sporobolomyces roseus	4.3	C	7.5	E	8.60	E
Syncephalastrum sp.	3.4	B	7.0	C	7.50	C
C.D.	0.521		0.933		0.758	

A: Homogenous intermingling grown, B : Pathogen overgrown by test fungus
C : Cessation of growth at the line of contact; D : Clear zone of inhibition;
E : Test fungus overgrown by pathogen.

a) *Geotrichum candidum*

Alternaria alternata, Aspergillus flavus, A. niger, A. aculeatus, A. terreus, Fusarium solani, F. oxysporum, Pythium aphanidermatum and *Syncephalastrum* sp. inhibited the pathogen by overgrowth (B). *Penicillium notatum* and *P. wortmanii* inhibited the pathogen by a clear zone of inhibition (D). *Sporobolomyces roseus* and *Cryptococcus* sp. inhibited the pathogen by cessation of growth at the point of contact (C).

b) *Fusarium solani*

None of the epiphytes inhibited the pathogen by overgrowth (B). *Alternaria alternata, Aspergillus flavus, A. niger, Fusarium oxysporum, Pythium aphanidermatum, Syncephalastrum* sp. inhibited the pathogen by cessation of growth at the point of contact (C). *A. aculeatus, A. terreus, Pencillium notatum* and *P. wortmanii* showed a clear zone of inhibition against the pathogen (D). *Sporobolomyces roseus* and *Cryptococcus* sp. were overgrown by the pathogen (E). *F. solani* showed homogenous intermingling with *F. solani*.

c) *Rhizoctonia solani*

None of the epiphytes inhibited the pathogen by overgrowth (B). *Alternaria alternata, Aspergillus flavus, A. niger, Fusarium oxysporum, F. solani, Pythium aphanidermatum* and *Syncephalastrum* sp. inhibited the pathogen by cessation of growth at the point of contact (C), while *A. aculeatus, A. terreus, Penicillium notatum* and *P. wortmanii* inhibited by the formation of a clear zone of inhibition (D). *Cryptococcus* sp. and *Sporobolomyces roseus* were overgrown by pathogen (E).

From the above table it could be seen that *Aspergillus terreus* was successful in inhibiting the major pathogens of tomato viz., *G. candidum*, *F. solani* and *R. solani*.

4.3.4 Selection of the superior antagonist

Aspergillus spp. obtained as superior antagonists were not used due to their potential to produce aflatoxins. Since *Gliocladium virens* and *Trichoderma harzianum* obtained from brinjal showed promising results, they were also tested against the pathogens of chilli and tomato along with the check, *T. viride*. Their mean colony diameter and interaction types is given in the table given below (Table 4).

Table 4 Mean colony diameter of the pathogens of chilli and tomato and their interaction types with the promising antagonists of brinjal

Epiphytes tested	*C. capsici*		*G. candidum*		*R. solani*		*F. solani*	
	colony diameter (cm)	Int. type	colony diameter (cm)	Int. type	colony diameter (cm)	Int. type	colony diameter (cm)	Int. type
G. virens	2.0	C	2.06	B	4.6	B	3.0	B
T. harzianum	2.0	C	2.62	B	4.6	B	2.6	B
T. viride (Check)	2.67	B	2.34	B	5.1	B	3.4	B
Control	8.5	-	7.70	-	9.0	-	9.0	-
CD	0.703		1.636		1.189		0.868	

B : Pathogen overgrown by test fungus C : Cessation of growth at the line of contact

The results showed that all the antagonists were capable of inhibiting the growth of the pathogens of chilli and brinjal.

G. virens inhibited *C. capsici* by cessation of growth at the point of contact (C) (Plate 13). *T. harzianum* inhibited the *C. capsici* by cessation of growth at the point of contact (C) (Plate 14), while *T. viride* used as check controlled *C. capsici* by overgrowth (B). The extent of inhibition shown by *T. harzianum*, *G. virens* and *T. viride* were on par.

Against *Geotrichum candidum* all the antagonists inhibited the pathogen by overgrowth (B). The extent of inhibition shown by them were statistically on par (Plate 15, 16).

R. solani was inhibited by all the three antagonists by overgrowth (B). The effect of all the three antagonists were on par (Plate 17, 18).

Fusarium solani was inhibited by overgrowth (B). Though *T. harzianum* recorded the maximum inhibition, all the antagonists were on par in their inhibition of the pathogen (Plate 19, 20).

Since *T. harzianum* and *G. virens* also inhibited the pathogens of brinjal as well as chilli and tomato to similar levels with the check viz., *T. viride* these were taken up for further studies.

4.3.4.1 Cultural characteristics

a) Gliocladium virens

The colony was fast growing, attained 9 cm in 6 days on PDA at 28 ± 1^{0}C. The colony was white to light green initially and turned dark green by third day. Plenty of aerial hyphae was noticed. Phialides were upto three in number,

Plate 13 Colony interaction showing cessation of growth at the point of contact between *G. virens* and *C. capsici*

Plate 14 Colony interaction showing cessation of growth at the point of contact between *T. harzianum* and *C. capsici*

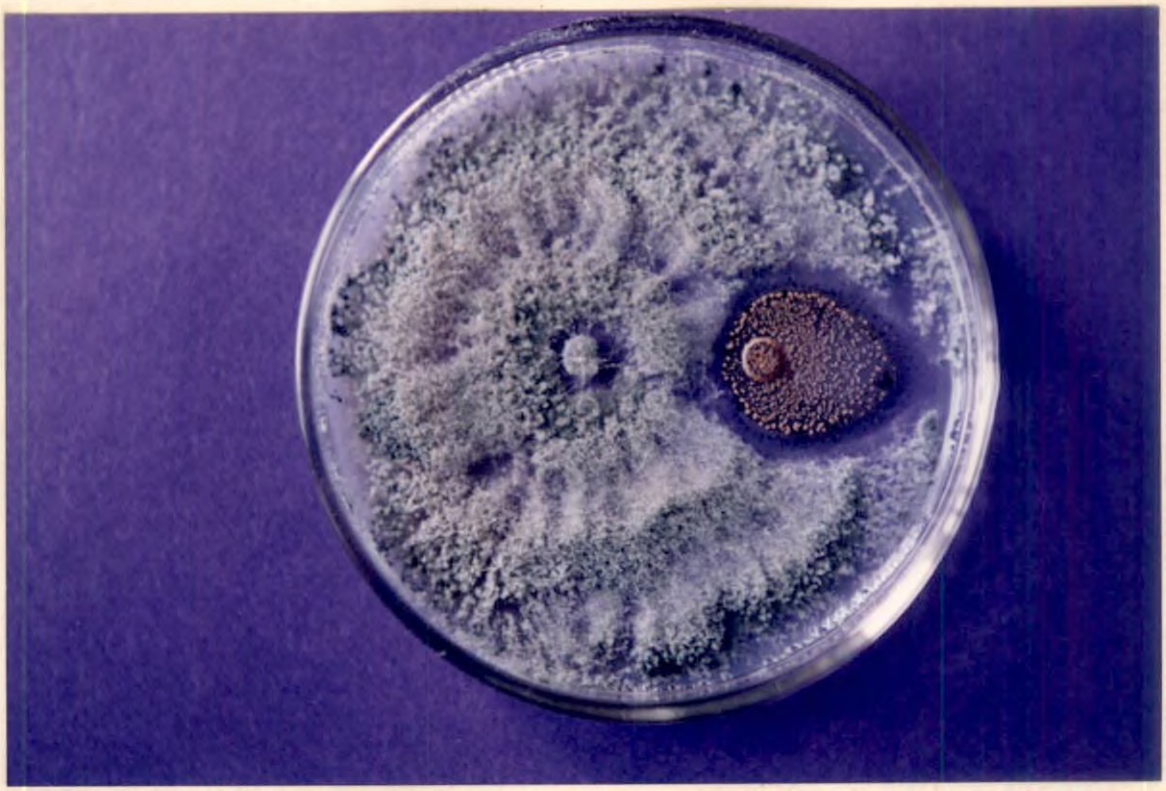
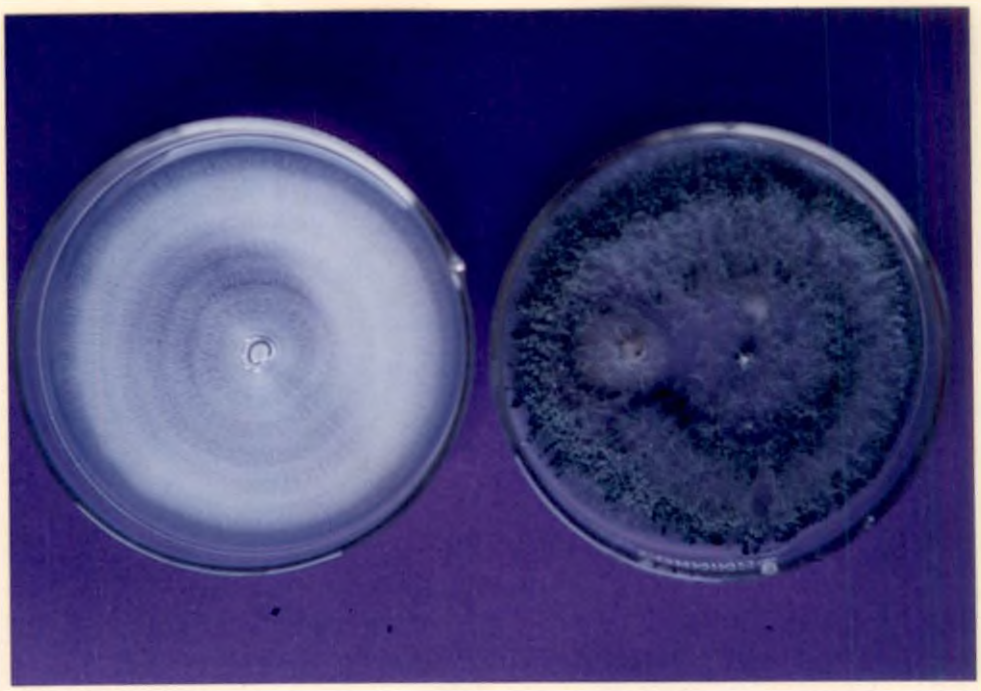


Plate 15 Colony interaction between *Gliocladium virens* and *Geotrichum candidum* showing overgrowth

Left : Control (*G. candidum*)
Right : *G. candidum* x *G. virens*

Plate 16 Colony interaction between *T. harzianum* and *G. candidum* showing overgrowth

Left : Control (*G. candidum*)
Right : *G. candidum* x *T. harzianum*



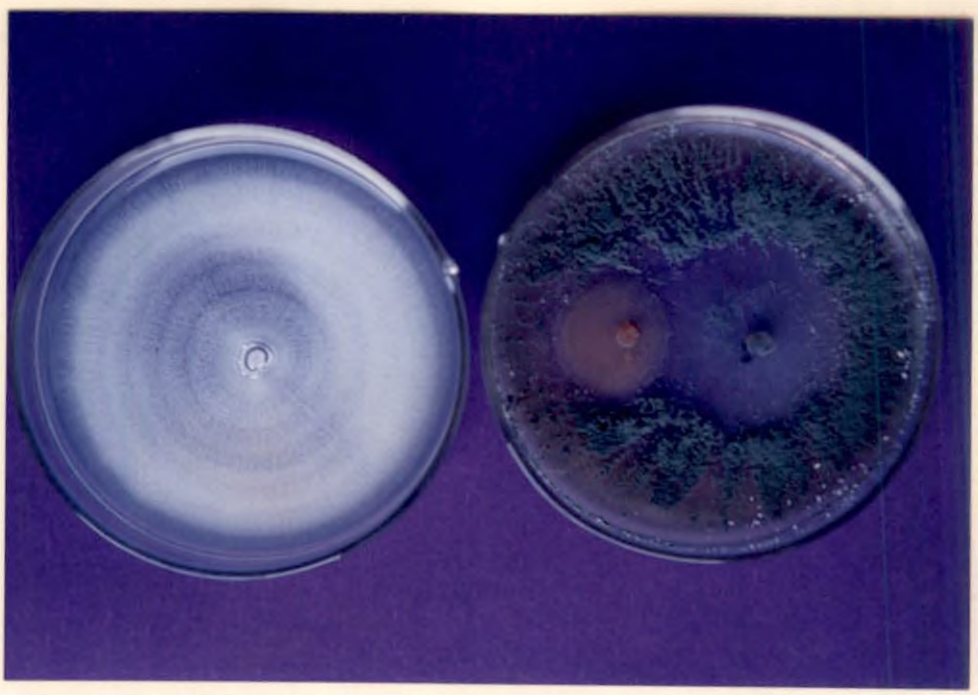


Plate 17 Colony interaction between *G. virens* and *R. solani* showing overgrowth

Left : Control (*R. solani*)
Right : *R. solani* x *G. virens*

Plate 18 Colony interaction between *T. harzianum* and *R. solani* showing overgrowth

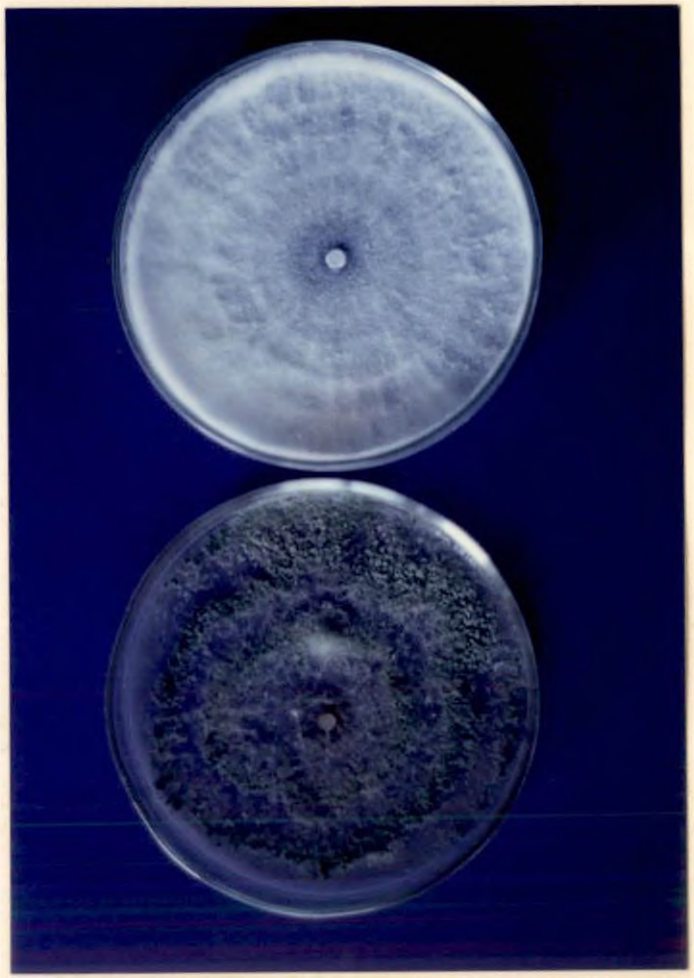
Left : Control (*R. solani*)
Right : *R. solani* x *T. harzianum*

Plate 19 Colony interaction between *G. virens* and *F. solani* showing overgrowth

Left : Control (*F. solani*)
Right : *F. solani* x *G. virens*

Plate 20 Colony interaction between *T. harzianum* and *F. solani* showing overgrowth

Left : Control (*F. solani*)
Right : *F. solani* x *T. harzianum*



long, appressed and bearing one large drop of green conidia on each whorl. They measured 7 - 13 x 1.8 - 2.5 µm. Conidia were short, ellipsoidal, smooth walled and measured 4.5 - 6 x 3.5 - 4 µm (Plate 21, Fig. 2).

The culture was identified as *Gliocladium virens* based on the above cultural characters and conidial morphology.

b) Trichoderma harzianum

The colony was fast growing attained 9 cm in 6 days, on PDA at 28 ± 1^0 C. The colony was initially light green and later turned dark green . Phialides were upto five in number, short, narrow at base with sharp pointed neck. They measured 5 - 7 x 3 - 4 µm. The conidia are smooth, globose and subglobose conidiophores. The conidia measured 2.8 - 3.2 x 2.5 - 2.8 µm (Plate 22, Fig. 3).

Based on the cultural characteristics the fungus was identified as *T. harzianum*.

4.4 Studies on mycoparasitism

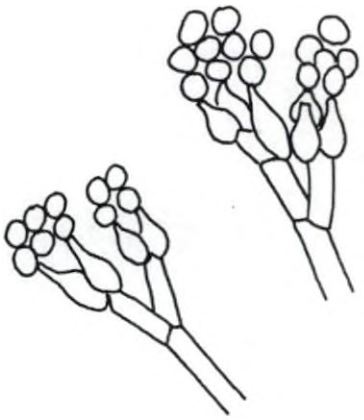
The result of the studies on the mechanism of mycoparasitism has been summarized in the table below (Table 5).

Table 5 Mechanism of mycoparasitism of *G. virens* and *T. harzianum* against pathogens of brinjal, chilli and tomato

Host	Pathogen	*G. virens*	*T. harzianum*
Brinjal	*P. vexans*	Coiling and vacuolation	Coiling and vacuolation
Chilli	*C. capsici*	Coiling and granulation	Formation of infection pegs
Tomato	*R. solani*	Granulation of mycelia	Coiling and vacuolation
Tomato	*G. candidum*	Disintegration of mycelia	Disintegration of mycelia
Tomato	*F. solani*	Coiling and penetration	Coiling and penetration

Fig. 2 Conidiophore and conidia of *Gliocladium virens*

Fig. 3 Conidiophore and conidia of *Trichoderma harzianum*



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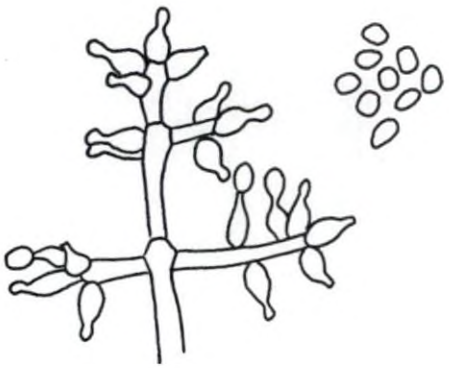

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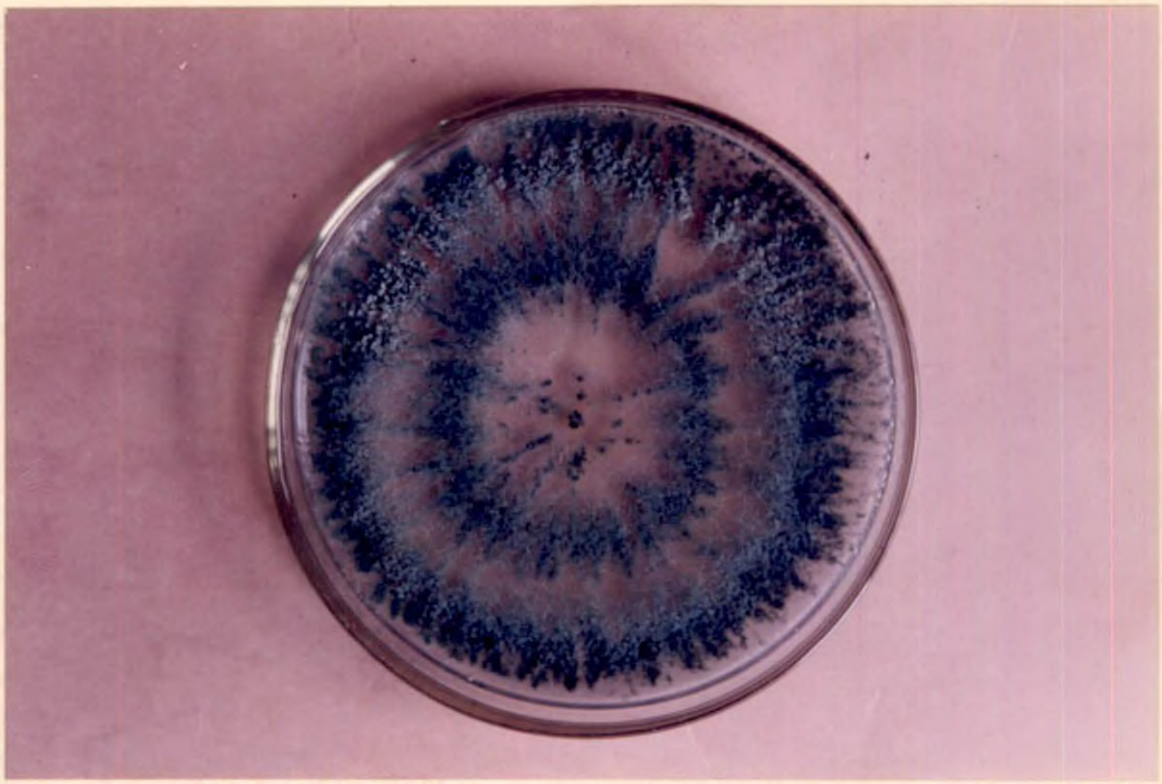
Plate 21 Growth of *G. virens* on PDA seven days after inoculation

Plate 22 Growth of *T. harzianum* on PDA seven days after inoculation



4.4.1 Mechanism of mycoparasitism

a) *Gliocladium virens*

In general coiling was noticed with *C. capsici* (Fig. 4), *P. vexans* and *F. solani*. The coiling accompanied by granulation and penetration was noticed with *C. capsici* and *F. solani* respectively. *P. vexans* and *R. solani* recorded only coiling and granulation of mycelia (Fig. 5). Disintegration was noticed with *Geotrichum candidum* only.

b) *Trichoderma harzianum*

Coiling and vacuolation of the fungal hyphae were noticed with *P. vexans* (Fig. 6) and *R. solani* (Fig. 7). *C. capsici* showed formation of infection pegs while disintegration of mycelia and coiling and penetration was recorded by *G. candidum* and *F. solani* respectively.

4.5 *In vivo* testing of the antagonist in the laboratory

4.5.1 Brinjal

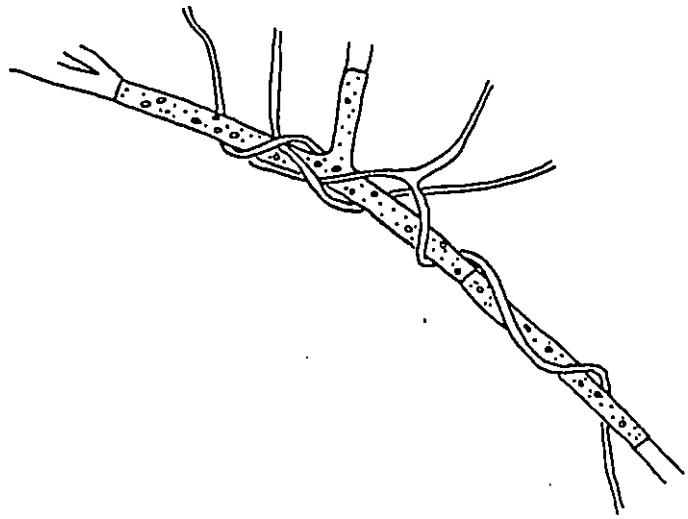
The results of the *in vivo* testing of the antagonist is summarized in the table below (Table 6).

Table 6 Effect of *G. virens* and *T. harzianum* on brinjal fruit (long variety) inoculated with the pathogen *P. vexans* (four and eight days of storage).

Days	Control (pathogen alone)	Pathogen + *G. virens*	Pathogen + *T. harzianum*
4 days	At the point of inoculation the colour turned from green to light brown. The lesion measured 2.5 cm in diameter and the tissues showed shrivelling.	Light brown depressed lesions of 1 cm diameter were seen.	Light brown depressed lesions of 1 cm diameter were seen.
8 days	Complete rotting occur. The tissues turned brown. Mycelial growth of pathogen was also seen.	The colour turned brown. Lesions measured 2.5 cm in diameter.	Lesions were 2.5 cm in diameter. The tissues started rotting.

Fig. 4 Coiling and granulation of hypha of *Colletotrichum capsici* by *G. virens*

Fig. 5 Granulation of mycelia of *Rhizoctonia solani* by *G. virens*



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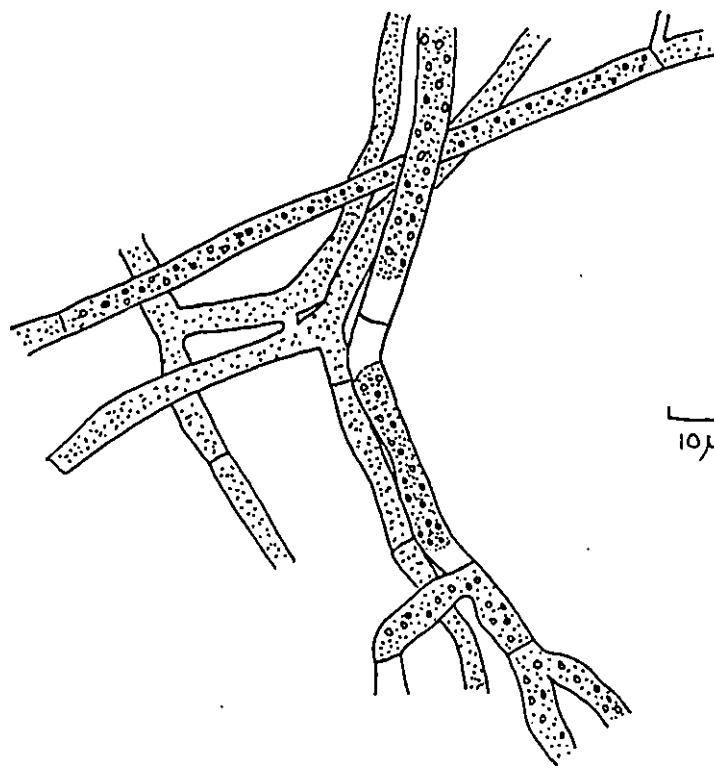

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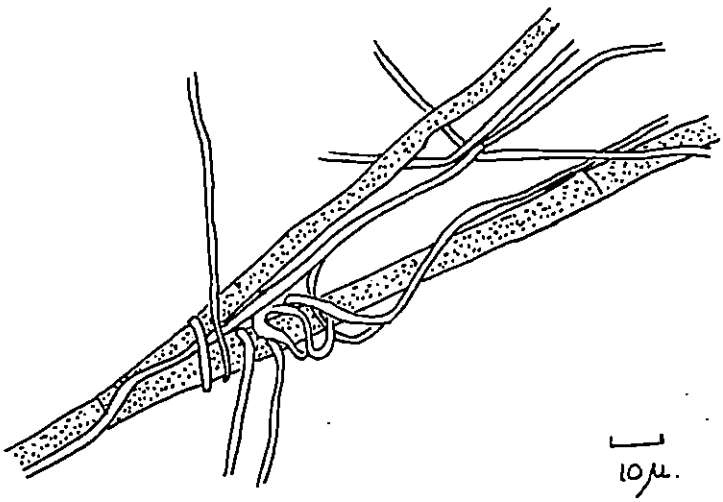
Fig. 6 Coiling and vacculation of hypha of *Phomopsis vexans* by *T. harzianum*

Fig. 7 Coiling and vacculation of hypha of *R. solani* by *T. harzianum*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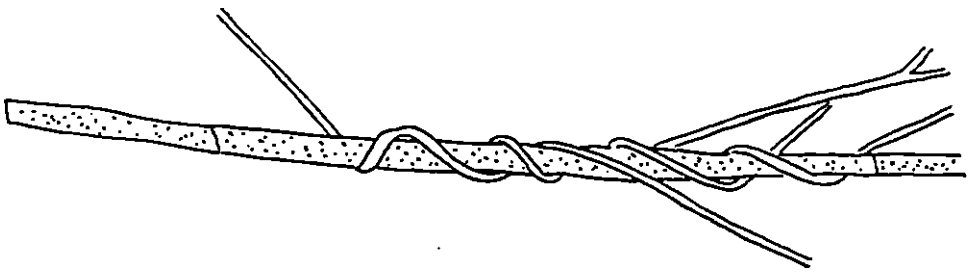


Brinjal fruits inoculated with the pathogen and dipped in the suspension of antagonistic fungi namely *G. virens* and *T. harzianum* did not show much difference in lesions, colour and development. The development of lesion was gradual when compared with the control. The control fruits showed complete rotting by the eighth day of incubation.

4.5.2 Chilli

The results of the *in vivo* testing of the antagonist is summarized in the table below (Table 7).

Table 7 Effect of *G. virens* and *T. harzianum* on chilli fruit inoculated with the pathogen *C. capsici* (four and eight days of storage)

Days	Control (pathogen alone)	Pathogen + *G. virens*	Pathogen + *T. harzianum*
4 days	Lesion turned black and measured 2.5 cm in diameter. This was completely covered by acervuli.	Light brown coloured lesion along the point of injury. Acervuli formation was not noticed.	Light brown coloured lesion along the point of injury. Acervuli formation was not noticed.
8 days	The lesions turned black and measured 2.5 cm in diameter and completely covered by acervuli.	One or two acervuli developed on the central portion and lesion measured 1.5 cm in diameter.	One or two acervuli developed on the central portion and lesion measured one cm in diameter.

The chilli fruits inoculated with *C. capsici* and dipped in suspension of the antagonists namely *G. virens* and *T. harzianum* recorded the following changes.

On the fourth day of incubation, light brown coloured lesions were seen in both cases at the point of inoculation without any acervuli formation, but on

the eighth day of inoculation, a slight difference was noticed. Fruits treated with *G. virens* recorded the lesion diameter of 1.5 cm and a few acervuli but chilli fruits treated with *T. harzianum* the lesion development was less (1 cm) and with only one or two acervuli.

4.5.3 Tomato

4.5.3.1 The results of the *in vivo* testing of the antagonists viz., *G. virens* and *T. harzianum* with the pathogen *Geotrichum candidum*

The results of the *in vivo* testing of the antagonist is summarized in the table below (Table 8).

Table 8 Effect of *G. virens* and *T. harzianum* on tomato fruit inoculated with the pathogen *Geotrichum candidum* (four and eight days of storage).

Days	Control (pathogen alone)	Pathogen + *G. virens*	Pathogen + *T. harziamum*
4 days	The skin around the point of inoculation was ruptured and a watery ooze occurred.	Tissues rotted around the point of inoculation	Tissues rotted around the point of inoculation
8 days	The fruit developed cracks and white mycelial growth along the crack was visible. There was a sour fruity order and heavy watery ooze followed.	Skin showed splitting and greenish growth of *G. virens* was noticed along the split surface.	Only slight rotting at the point of inoculation.

Tomato fruit inoculated with *G. candidum* and dipped in *G. virens* showed rotting around the point of inoculation by four days, and by the eighth day the skin showed splitting and was partially covered by greenish growth of the antagonists, *G. virens* (Plate 23).

Plate 23 Symptoms of *G. virens* on tomato fruits eight days after inoculation

4.5.3.2 The results of the *in vivo* testing of the antagonists viz., *G. virens* and *T. harzianum* with the pathogen *Fusarium solani*

The results of the *in vivo* testing of the antagonist is summarized in the table below (Table 9).

Table 9 Effect of *G. virens* and *T. harzianum* on tomato fruit inoculated with the pathogen *F. solani* (four and eight days of storage).

Days	Control (pathogen alone)	Pathogen + *G. virens*	Pathogen + *T. harzianum*
4 days	White mycelial growth seen at the point of inoculation. The area around it was rotten and soft.	There was a slight rot and depression at the point of inoculation.	There was a slight rot and depression at the point of inoculation. The fruit was firm
8 days	Fruit was in a split and broken state. They was watery exudation and mycelial growth was visible along the split surface.	The fruit was in a split size. Greenish growth of mycelia and sporulation of *G. virens* was visible on the fruit.	The fruit was in a split state with a growth of *F. solani* visible along the split surface.

The tomato fruit inoculated with *F. solani* and treated with the antagonistic suspension of *G. virens*, profuse greenish growth of mycelia and sporulation by *G. virens* was observed after eight days of inoculation.

But on treatment of *T. harzianum* the fruit was firm for first four days and then showed slight splitting with growth of *F. solani*.

4.5.3.3 The results of the *in vivo* testing of the antagonists viz., *G. virens* and *T. harzianum* with the pathogen *Rhizoctonia solani*

The results of the *in vivo* testing of the antagonist is summarized in the table below (Table 10).

Table 10 Effect of *G. virens* and *T. harzianum* on tomato fruit inoculated with the pathogen *R. solani* (four and eight days of storage).

Days	Control (pathogen alone)	Pathogen + *G. virens*	Pathogen + *T. harzianum*
4 days	The point of inoculation had a depression and skin was slightly broken.	There was a depression at the point of inoculation. The fruit was firm.	Slight rot was observed at the point of inoculation. The fruit was firm.
8 days	The fruit was in a broken and split state. It was soft to touch and there was fouls smell. Heavy exudation of water followed.	Only a slight rot at the point of inoculation was visible. The fruit was firm.	Only a slight rot at the point of inoculation was visible. The fruit was firm.

Tomato fruits inoculated with *R. solani* and treated with the suspension of *G. virens*, only a slight rot was seen on the eighth day of inoculation.

On treatment with *T. harzianum* also, only a slight rot was observed at the point of inoculation and the fruit was firm.

The results of the studies show that with brinjal none of the antagonist were effective (Table 6). With chilli, *T. harzianum* was found to be effective (Table 7). In tomato with *G. candidum*, *T. harzianum* was found to be effective (Table 8). Both the mycoparasites were found to be ineffective on tomato inoculated with *F. solani* (Table 9). While on tomato inoculated with *R. solani*, *T. harzianum* was found to be effective (Table 10) in reducing the infection.

Based on the above observation *T. harzianum* was found to be effective for the management of post harvest rotting in tomatoes. and hence selected for further study.

4.4.6 Effect of carrier material on the viability and shelf life of the fungal antagonist (*T. harzianum*)

The average number of spores per field and their germination percentages on weekly basis is provided in the Table 11.

Table 11 Percentage of initial inoculum present and germination percentage of the spores in different carrier materials after storage

Days	Coirpith		Wheat flour		Charcoal		Talc	
	Percentage of initial inoculum present	Germination per cent	Percentage of initial inoculum present	Germination per cent	Percentage of initial inoculum present	Germination per cent	Percentage of initial inoculum present	Germination per cent
0	100.00	40.35	100.00	42.20	100.00	34.90	100.00	42.80
7	96.60	19.10	116.30	47.28	100.00	9.960	59.20	31.10
14	53.70	12.80	132.50	48.80	88.80	12.60	31.50	63.30
21	12.70	38.20	21.50	51.20	13.70	27.20	15.70	100.00
28	18.40	26.50	15.70	63.30	3.70	100.00	17.10	100.00
35	8.60	52.20	12.20	68.80	7.50	80.00	15.70	100.00
42	4.80	76.92	9.30	92.80	3.70	100.00	15.70	100.00

Wheat flour recorded a rise in the population. This continued upto 14 days and thereafter recorded a steep decline by 21 days and it then continued without much change till the end of 42 days. In charcoal, coirpith and talc the

decline was very fast upto 21 days and thereafter continued without much change till the end of 42 days (Fig. 8, Table 11).

Studies on the viability of spores showed that germination percentage reached maximum by 42 days of storage in the carrier materials namely coir pith, wheat flour, charcoal and talc. However with talc 100 per cent germination was noticed on 21 days of storage and continued till 42 days of storage. Based on the amount of initial inoculum remaining after 42 days and the uniformity shown in the germination (100 per cent), talc was found to be superior to the other carrier materials and hence taken up for further study (Fig. 9, Table 11).

4.6.1 Testing the viability of spores in talc based formulation (25 per cent)

The viable spore count of the propagules of the antagonist *T. harzianum* was observed and the following result obtained (Table 12).

Table 12 Viable spore count of *T. harzianum* in talc

Days	Count/g of formulation
0	1.5×10^7
7	1×10^6
14	16×10^5
21	14×10^5
28	12×10^5
35	10×10^5
42	11×10^5

The spore count was found to be uniform from 14^{th} day to the 42^{nd} day.

Fig. 8 Per cent variations in the population count in different carrier materials based on number of spores per microscopic field

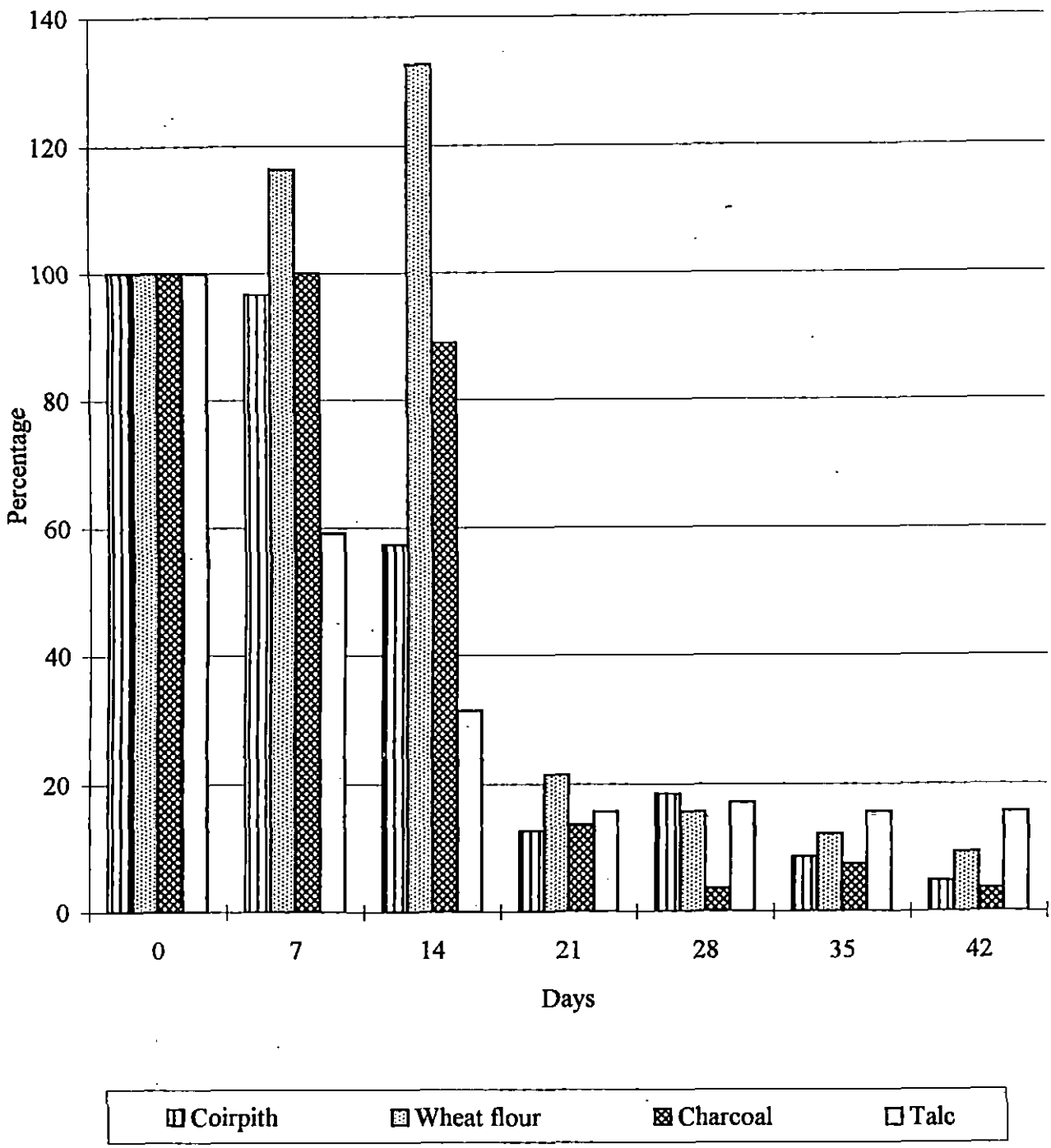
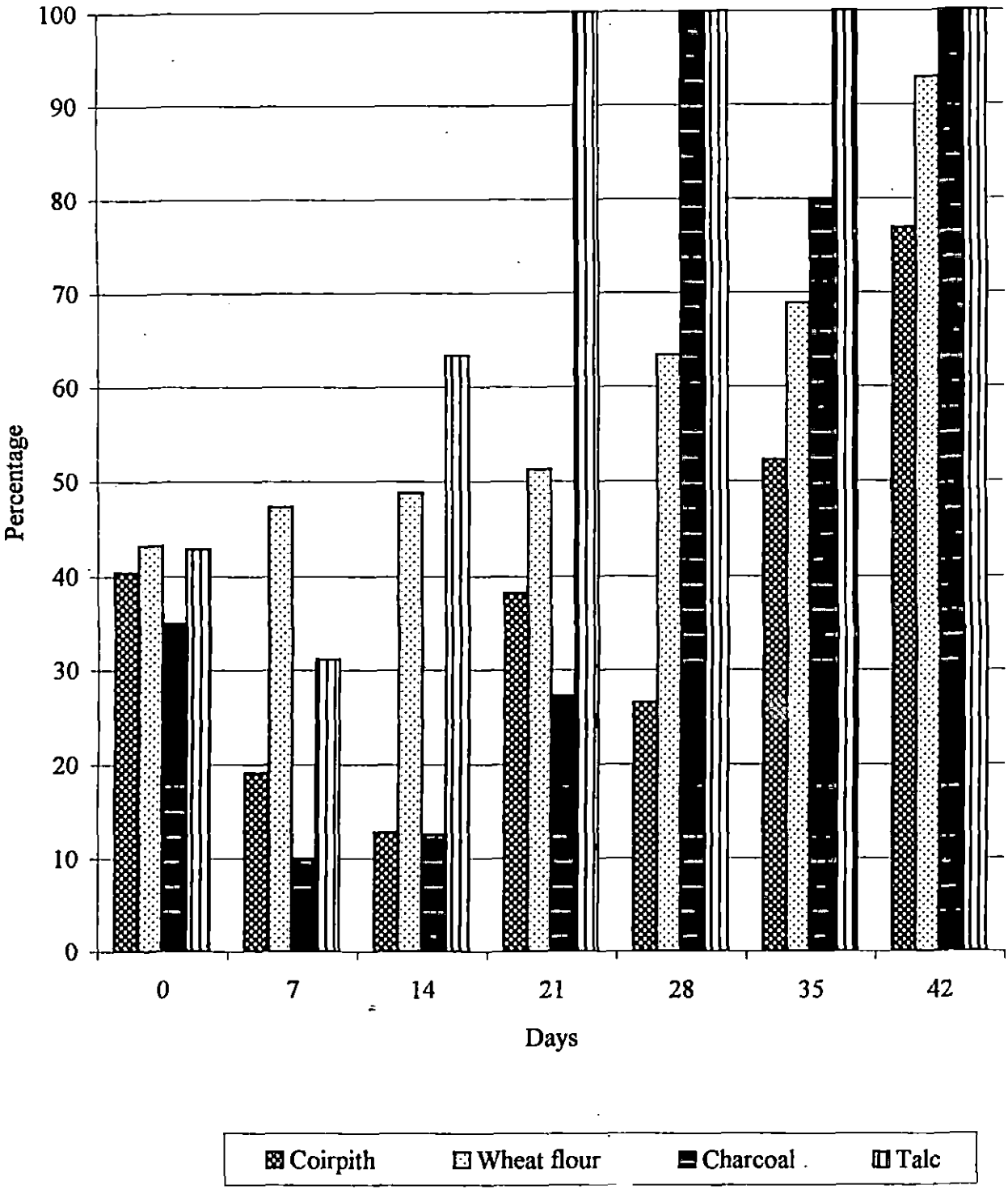


Fig. 9 Per cent variation in the germination count in different carrier materials based on number of spores per microscopic field



4.7 Comparative study of different methods of application of the antagonists (*T. harzianum*)

4.7.1 Effect of *T. harzianum* formulation on infection caused by *Phomopsis vexans* by methods of dipping and dusting

The results of the *in vivo* testing of the different methods of application of the antagonist is summarized in the table below (Table 13).

Table 13 Effect of different methods of application of the antagonistic formulation on brinjal fruits inoculated with *P. vexans* (Four and eight days after storage)

Days	Control (pathogen alone)	Dipping	Dusting
4 days	At the point of inoculation the colour turned from green to light brown. The lesion measured 2.5 cm in diameter and the tissues showed shrivelling.	Symptoms not developed.	Symptoms not developed.
8 days	Complete rotting caused. Tissues turned brown. Mycelial growth of pathogen was also seen.	Symptoms not developed.	Symptoms not developed.

Brinjal inoculated by *P. vexans* and treated with the formulation showed damage compared to control. In both cases, dipping and dusting, even after eight days no symptoms were observed. The control showed complete rotting by the eighth day of inoculation (Plate 24, 25)

Plate 24 Effect of the formulation (dusting) on the rot of brinjal caused by *P. vexans* (eight days after inoculation)

Plate 25 Effect of the formulation (dipping) on the rot of brinjal caused by *P. vexans* (eight days after inoculation)

4.7.2 Effect of *T. harzianum* formulation on infection caused by *Colletotrichum capsici* by methods of dipping and dusting

The results of the *in vivo* testing of the different methods of application of the antagonist is summarized in the table below (Table 14).

Table 14 Effect of different methods of application of the antagonistic formulation on chilli fruits inoculated with *C. capsici* (Four and eight days after storage).

Days	Control (pathogen alone)	Dipping	Dusting
4 days	Lesions turned black and measured 2.5 cm in diameter. This was completely covered by acervuli	The brown colouration was seen at the point of inoculation. Lesion of 0.5 cm diameter formed, no acervuli was seen	A brown colouration along the cut surface. Black pin head like acervuli were seen and lesions were of 1 cm diameter.
8 days	Lesions turned black and measured 2.5 cm in diameter. This was completely covered by acervuli	The soft rot type of symptom developed. Lesions of 2 cm diameter were noticed with pin head like acervuli	The soft rot type of symptom developed. Lesions of 2 cm diameter were noticed with pin head like acervuli

Chilli fruit inoculated with *C. capsici* and treated with formulation by both dipping and dusting did not control the effect of *C. capsici*. Lesions of two cm diameter and growth of acervuli were seen in both cases (Plate 26, 27)

4.7.3 Effect of *T. harzianum* formulation on tomato

4.7.3.1 Effect of *T. harzianum* formulation on tomato infected by *Geotrichum candidum* by methods of dipping and dusting

The results of the *in vivo* testing of the different methods of application of the antagonist is summarized in the table below (Table 15).

Plate 26 Effect of the formulation (dusting) on the rot of chilli caused by *C. capsici* (eight days after inoculation)

Plate 27 Effect of the formulation (dipping) on the rot of chilli caused by *C. capsici* (eight days after inoculation)

Table 15 Effect of different methods of application of the antagonistic formulation on tomato fruits inoculated with *G. candidum* (Four and eight days after storage).

Days	Control (pathogen alone)	Dipping	Dusting
4 days	The skin around the point of inoculation was ruptured and a watery ooze occurred.	Symptoms not produced.	Symptoms not produced.
8 days	The fruit developed cracks and white mycelial growth along the crack was visible. There was a sour fruity order and heavy watery ooze followed.	Slight initiation of rot on the fruit	Symptoms not produced.

Geotrichum candidum causing rot of tomatoes was well controlled by dusting. The rotting symptoms were not observed even on the eighth day of inoculation, while the control samples showed complete rotting.

With the dip treatment also no symptoms were observed on the fourth day. Slight initiation of rot was seen on the eighth day (Plate 28, 29)

4.7.3.2 Effect of *T. harzianum* formulation on tomato infected by *F. solani* by methods of dipping and dusting

The results of the *in vivo* testing of the different methods of application of the antagonist is summarized in the table below (Table 16).

Table 16 Effect of different methods of application of the antagonistic formulation on tomato fruits inoculated with *F. solani* (Four and eight days after storage).

Days	Control (pathogen alone)	Dipping	Dusting
4 days	White mycelial growth seen at the point of inoculation. The area around it was rotten and soft.	A slight rot initiated on the fruit with mild growth of mycelia visible.	Symptoms not produced.
8 days	Fruit was in a split and broken state. They was watery exudation and mycelial growth was visible along the split surface.	Fruit showed rotting and water loss.	Mild rotting initiated at the point of inoculation.

Plate 28 Effect of the formulation (dusting) on the rot of tomato caused by *G. candidum* (eight days after inoculation)

Plate 29 Effect of the formulation (dipping) on the rot of tomato caused by *G. candidum* (eight days after inoculation)

Fusarium solani causing rot of tomatoes was not controlled by dusting or dipping. By the eighth day of inoculation both the treatment showed symptoms of rotting (Plate 30, 31)

4.7.3.3 Effect of *T. harzianum* formulation on tomato infected by *R. solani* by methods of dipping and dusting

The results of the *in vivo* testing of the different methods of application of the antagonist is summarized in the table below (Table 17).

Table 17 Effect of different methods of application of the antagonistic formulation on tomato fruits inoculated with *R. solani* (Four and eight days after storage).

Days	Control (pathogen alone)	Dipping	Dusting
4 days	The point of inoculation had a depression and skin was slightly broken.	Mild rotting was initiated as a water soaked lesion at the point of inoculation.	Symptoms not produced.
8 days	The fruit was in a broken and split state. It was soft to touch and there was fouls smell. Heavy exudation of water followed.	Rotting increased accompanied by watery ooze.	Symptoms not produced.

R. solani causing rot of tomatoes was controlled by dusting. Dusting was found to be more effective than dipping. No symptoms were observed in dusting even after eight days of inoculation. In the case of dipping mild rotting was observed after eight days of inoculation (Plate 32, 33)

Based on the study (Table 13, 14, 15, 16 and 17) dusting of *T. harzianum* was found to be superior and hence selected as the method of application.

Plate 30 Effect of the formulation (dusting) on the rot of tomato caused by *F. solani* (eight days after inoculation)

Plate 31 Effect of the formulation (dipping) on the rot of tomato caused by *F. solani* (eight days after inoculation)

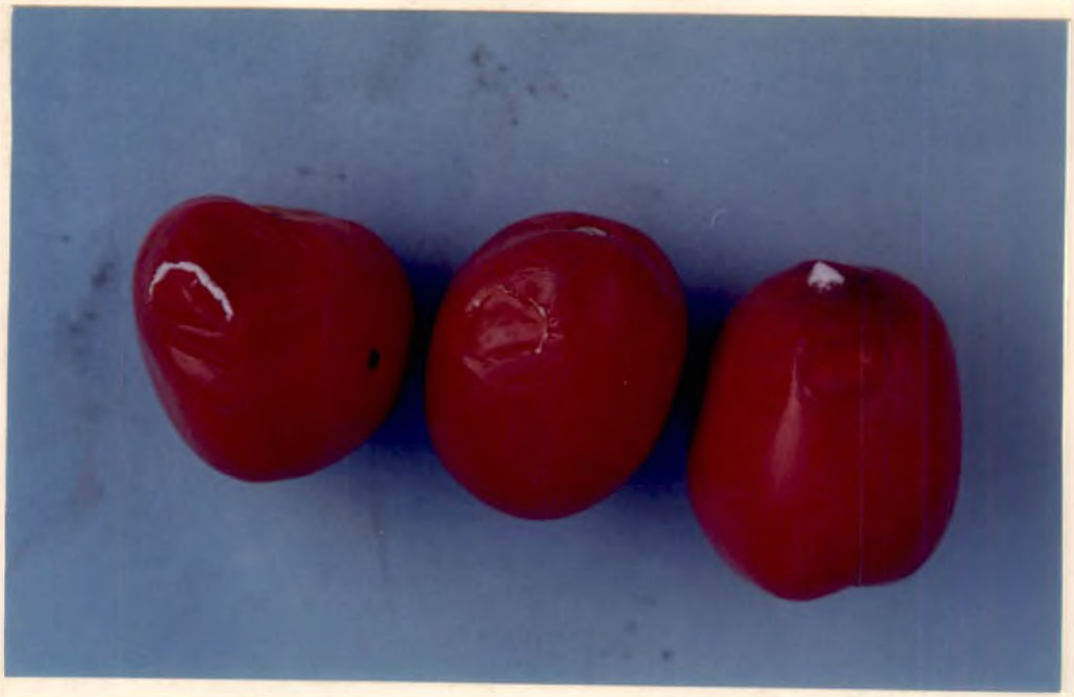


Plate 32 Effect of the formulation (dusting) on the rot of tomato caused by *R. solani* (eight days after inoculation)

Plate 33 Effect of the formulation (dipping) on the rot of tomato caused by *R. solani* (eight days after inoculation)

DISCUSSION

5. DISCUSSION

Fungi are mainly responsible for the spoilage of vegetable. The transit and storage atmosphere plays a key role in development of symptoms. The spores present on the surface in a latent form may develop lesions around injuries, sustained during the process of harvesting, transporting and storage. The intensity of symptoms may vary depending upon the climatic factors and the storage practices.

The common fungi associated with the storage of brinjal, chilli and tomato are :

Brinjal

1. *Phomopsis vexans*
2. *Phytophthora capsici*
3. *Pythium aphanidermatum*

Chilli

1. *Colletotrichum capsici*
2. *Phytophthora capsici*
3. *Alternaria alternata*
4. *Botrytis cinerea*

Tomato

1. *Geotrichum candidum*
2. *Rhizoctonia solani*
3. *Fusarium solani*
4. *Rhizopus stolonifer*
5. *Pythium aphanidermatum*
6. *Alternaria alternata*

In brinjal *Phomopsis vexans* causes considerable damage. The incidence of the disease has been reported from different parts of India (Pawar and Patel, (1957); Bilgrami *et al.* (1979); Ashwanikumar *et al.* (1986), Dasgupta and Mandal (1989); John (1991) and Reeny (1995). The infection appears anywhere on the fruit, mostly at the pedicel end. Symptoms may vary depending on the relative humidity of the atmosphere. Reeny (1995) has reported considerable damage from April to December when the relative humidity and temperature ranged from 75-95 per cent and 25-35^{0}C respectively.

P. capsici is found to be the next major pathogen infecting the brinjal fruits. Storage rot due to *Phytophthora* in causing soft rots is already reported by Jain *et al.* (1982). Complete rotting is caused in three days on artificial inoculation. This disease is maximum during rainy seasons as observed by Critopoulose (1954). Another important pathogen associated with rotting of brinjal is *P. aphanidermatum*. It is also common during rainy seasons and is known to cause rotting in other vegetables like ashgourd, potato, carrot, yams (Dasgupta and Mandal, 1989) besides brinjal and tomato.

In chilli, *C. capsici*, is the most important pathogen. The fungus causes ripe rot in fruits and die back in plants. The fruits nearing maturity are badly damaged. Ripe rot is more conspicuous and continues to damage fruits during transit and storage. Chaudhury (1957) has reported a loss of 30 per cent from Assam. Mishra and Mahmood (1960) reported an optimum temperature of 28-30^{0}C with relative humidity of 90 per cent to be ideal for infection and growth. *C. acutatum* has also been reported to cause fruit rot in Punjab (Kaur and Singh, 1990).

Alternaria alternata has also been recorded on chilli. McDonald and de Wildt (1980) have reported 16.2 per cent of chilli fruits lost due to *A. alternata* in wholesale market and found the infection to be correlated with the high temperature during storage. Similar observation has also been made by Sharma and Sumbali (1993).

The soft rot in chilli caused by *Botrytis cinerea* recorded in this study has also been recorded earlier (Lopez *et al.*, 1986). They have stated it to be a soil borne disease affecting the fruits which are in close, contact with the soil. The pathogen gains entry through the injuries caused during harvesting.

In tomato considerable damage is caused by *G. candidum*. This is an important pathogen affecting tomatoes. The fruit is damaged by cracking followed by profuse leaking. The leaking spoils the consistency and fruits get misshapen and shrunken. It is recorded to have caused losses of thousands of tonnes of ripe tomatoes in the US (Butler, 1960). It is considered as a wound pathogen. The spread of the pathogen in the market is by the fruit fly, *Drosophila melanogaster* (Butler, 1961 and Butler and Bracker, 1963). In India, Thapa and Sharma (1976) have also attributed a loss of about 15 per cent of tomatoes due to *G. candidum* alone.

Fusarium rot caused by *F. solani* is the next important pathogen. The fungus also causes severe losses in markets. The infection is more on injured tomatoes. Maximum rotting is observed at 28^{0}C to 30^{0}C (Garg and Gupta, 1979). Loss due to *F. solani* in tomatoes has also been reported by Thomas *et al.* (1981). The warm and humid conditions during transit and storage is very conducive for growth and multiplication (Thapa and Sharma, 1976). Reeny (1995)

The soft rot due to *R. solani* is also equally important. This fungus grains entry from the soil at the time of harvesting (Thapa and Sharma, 1976). The fungus is reported from several parts of the world (Thomas *et al.*, 1981). The studies conducted by Reeny (1995) at Vellayani reported *R. solani* to be prevalent during the rainy periods of the year.

R. stolonifer has also been observed to infect tomatoes. This organism is reported to cause soft rots by McColloch *et al.* (1968) in the United States. From India it is reported from Uttar Pradesh (Bilgrami *et al.*, 1979) and from Vellayani (Reeny, 1995).

The infection occurs mostly at the cracked areas and the spread is facilitated by the fruit fly *D. melanogaster* (Butler and Braker, 1963). Sharma and Sumbali (1993) have also reported *R. stolonifer* infection under conditions of high temperature and humidity.

A. alternata is also an important pathogen infecting the tomatoes. McColloch and Worthington (1952) have stated that the pathogen is carried from the field to the store house. Uma (1984) has recorded *A. alternata* as a weak pathogen infecting only wounded and weak fruits. Widespread damage to tomatoes has also been recorded by Zitter and Wien (1984).

Studies on the epiphytes of fruits have yielded several fungi. It is well recognized that the fruit surface constitutes distinct microhabitat which is inhabited by a varied assemblage of saprophytic and parasitic microorganisms and there exists complex relationships between the organisms. The quantitative and qualitative nature of the microorganisms on the fruit surface are influenced to a large extent by the plant species, nature of fruit surface, age of plant,

mycoflora obtained showed that brinjal harboured a rich crop of fungi (18 numbers) followed by tomato (14 numbers) and chilli (12 numbers) Sinha (1971) has reported that the highest total number of microorganisms were recorded from chilly leaves and invariably the number of organisms increased with increasing leaf area. It can hence be said that brinjal fruit being largest in size yielded the maximum fungal colonies.

Some of the epiphytes like *Aspergillus terreus, A. niger, Cryptococcus* sp., *Fusarium solani, Pencillium wortmanii* were common to all the three vegetables studied. *Aspergillus* spp. were found to be most abundant. Similar results have been obtained by Bhagyaraj *et al.* (1962). Some epiphytes were specific to particular fruits. Thus brinjal harboured fungi like *Aspergillus ochraceous, Alternaria solani, Botryodiplodia theobromae, Gliocladium virens, Helminthosporium* sp., *Pestalotiopsis palmarum, Phomopsis vexans, Rhizopus* sp. and *Trichoderma harzianum* while *Cladosporium cladosporioides, Curvularia lunata, Fusarium tricinctum, Penicillium oxalicum, Stegmina* sp. were specific for chilli and *F. oxysporum, G. candidum* and *Syncephalastrum* sp. specific to tomato. Host specificity of fungi has already been reported by Singh and Sinha (1962) and Sinha (1971). Bhagyaraj *et al.* (1962) have stated that specificity of the fungi to certain crops is due to the specific nature of nutrients and excretions of plant at a particular stage.

Pathogenic fungi like *P. aphanidermatum, F. solani, A. alternata, Rhizopus* sp., *G. candidum, P. vexans* were also obtained as epiphytes.

Studies on the epiphytic mycoflora of fruits have been limited mainly on apple and strawberry. Beneke *et al.* (1954) have obtained *Cladosporium* sp.,

Aspergillus sp., *Trichoderma* and *Mucor* sp., from ripe strawberry fruits. Similarly Lowings (1950) also obtained *Mucor piriformis, B. cinerea, C. cladosporioides, Penicillium* sp., *Trichoderma* sp. and *Kloeckera* sp. from strawberry fruits. Tasca and Verga (1978) studied the mycoflora isolated from fruit and seed of red pepper and obtained *Rhizopus nigricans* most frequently. Bisiach (1985) obtained *Aureobasidium* sp. and *Cladosporium* sp. from the surface of grapes which could control gray mould fungi (*Botrytis cinerea*) of grapes. Reeny (1995) also obtained *T. viride* from the leaf washings of brinjal, chilli and tomato and observed to be effective in suppressing the rotting of these vegetables. Wolfgang *et al.* (1997) studied the fruit and leaf washings of apple and obtained *Rhodotorula glutinis, Aureobasidium pullulans* and *Bacillus subtilis* strains to be effective against many pathogens of apple.

The epiphytic fungi isolated were all tested *in vivo* for their antagonism against the major pathogens *viz.*, *P. vexans* (brinjal), *C. capsici* (chilli) and *G. candidum, R. solani* and *F. solani* (tomato). *Aspergillus terreus, Gliocladium virens* and *Trichoderma harzianum* showed antagonistic activity against major pathogens tested. *A. terreus* was not considered for the studies because of it involvement in the production of mycotoxins (Miller, 1961). Reeny (1995) also did not take up *A. terreus* in her studies due to similar reasons. The study was hence, limited to *G. virens* and *T. harzianum*.

Several reports on the occurrence of *Trichoderma* and *Gliocladium* (Boosalis, 1964 ; Tronsmo and Dennis, 1977; Hadar *et al.*, 1979; Elad *et al.*, 1980) and bacteria (Gutter and Littauer, 1953; Pusey *et al.*, 1986) to be antagonistic to a wide range of pathogens are available. These

microorganisms have been successfully utilised against a wide range of soil borne fungi, foliar and postharvest diseases of fruits and vegetables.

Mechanism of inhibition was also studied in detail. It is observed that *Phomopsis vexans* is inhibited by *G. virens* and *T. harzianum* by coiling and vacuolation. Gindart *et al.* (1983) and Ramert (1983) have reported *G. roseum* and *T. harzianum* to inhibit *P. sclerotioides* by mechanism of penetration and coiling.

The pathogen of chilli *viz.*, *C. capsici* is also inhibited by *G. virens* by coiling and granulation while *T. harzianum* inhibited *C. capsici* by the formation of infection pegs. Production of infection pegs by *T. harzianum* against *Macrophomina phaseolina* has been reported by Elad *et al.* (1986). Reeny (1995) has recorded parasitism of *C. capsici* by *T. viride* through penetration.

Similarly in tomato *viz.*, *G. candidum* is inhibited by both *G. virens* and *T. harzianum* by disintegration of fungal hyphae. *T. viride* and *T. harzianum* are reported to affect the mycohost by disintegration of mycelia (Gokulapalan, 1989 and Reeny, 1995).

Another pathogen of tomato viz., *R. solani* is inhibited by *G. virens* through granulation of mycelia while *T. harzianum* inhibited by coiling and vacuolation. *R. solani* is also reported to be inhibited by extracellular enzymes of *T. harzianum* (Hadar *et al.*, 1979). Similar results have also been obtained by Lewis and Papavizas (1984) and Tu and Vaartaja (1981). Gokulapalan (1989) and Reeny (1995) have reported *R. solani* to be inhibited by coiling and penetration by *T. viride*.

Similarly *F. solani* is inhibited by both *G. virens* and *T. harzianum* the mechanism being coiling and penetration. A similar mechanism of inhibition by *T. viride* has been reported by Reeny (1995) on *F. solani*.

Thus the role of the *G. virens* and *T. harzianum* is well established against major pathogens of brinjal (*P. vexans*), chilli (*C. capsici*) and tomato (*G. candidum, R. solani and F. solani*).

G. virens and *T. harzianum* were also tested *in vivo* for their ability to suppress common fruit pathogens of brinjal, chilli and tomato. The dip treatment of tomatoes in *G. virens* suspension resulted in the infection of dipped fruits by *G. virens*. It has been reported by Sumbali and Malhothra (1989) that *G. roseum* causes hard rot in carrot. Menzies (1993) reported that *T. viride* is also pathogenic on tomato seedlings under the laboratory conditions. However, *T. harzianum* was found to be an efficient antagonist against *G. candidum, R. solani* in tomatoes and *P. vexans* in brinjal.

Various *Trichoderma* spp. have been used to protect fruits against the postharvest diseases. Thus Tronsmo and Dennis (1977) successfully controlled strawberry rot by *Botrytis cinerea* using *Trichoderma* spp. *T. pseudokoningii* has also been utilized to suppress *B. cinerea* in apple (Tronsmo and Raa, 1977; Tronsmo and Ystaas, 1980). Similarly Bisiach *et al.* (1985) successfully controlled *B. cinerea* on grapes using five different strains of *T. harzianum*.

The result of the above study thus indicated the possibility of *T. harzianum* to be formulated and used as a biocontrol agent for the postharvest disease of vegetables.

The study on the germination percentage and viability of the antagonist viz., *T. harzianum* in the different carrier materials revealed that the conidial

count increased up to 14 days of storage and thereafter registered a decline in the case of wheat flour. In the other carrier materials *viz.*, coir pith, charcoal and talc there was a steady decline in the number of conidia from the beginning. In talc the viable spore count was found to be 1.1 x 10^6 cfu/g at the end of 42 days. A similar observation was recorded by Prasad *et al.* (1997), in *T. harzianum* wherein an increase in the number of propagules in wheat bran and pesta granules for first 30 days and then a decline at room temperature was recorded. A gradual decline in conidial count was observed in the case of talc and gypsum. Susha (1997) also observed an increase in the number of conidia of *C. gloeosporioides* and *F. equisetti* up to 20 days and then a gradual decline in the carrier material like rice bran, coir pith, wheat bran and vermiculite. In the present study, a maximum of 15.7 per cent viable conidia was contained in talc after 42 days of storage. With studies on the shelf life of *T. harzianum* on powdered talc based formulation, Sankar and Jayarajan (1996) observed that conidial population was slightly reduced up to 75 days and even after 120 days of storage, about 25 per cent of the propagules remained viable. Similar findings have also been recorded by Papavizas *et al.* (1984) in *Trichoderma viride* mixed with Pyrax (anhydrous aluminium silicate). Burges (1998) stated that propagules of the antagonists have greater shelf life in talc as the talc particles have the property of reflecting the harmful UV rays which affect the viability of the spores of the antagonists.

The effect of different methods of applications (dipping and dusting) of the antagonistic formulation showed that on brinjal and tomato the dusting treatment was effective in controlling the respective disease upto 4 days.

During this period the control treatments gave clear symptoms of the disease. The dipping method prevented the symptom development in the case of brinjal even on 8 days of storage. In tomato inoculated with *G. candidum* , symptom development was prevented only up to 4 days and slight symptoms were visible by 8 days. Tomatoes inoculated with *R. solani* and *F. solani* did not show any control by the dipping treatment by 4 day of treatment.

Few attempts of this kind have been carried out on vegetables. Most of the works have been done on fruits such as strawberry and apples. Tronsmo and Dennis (1977) controlled *Botrytis* rot of strawberry by spraying *Trichoderma* spp. on the matured fruits. Similarly Pusey and Wilson (1984) have stated that dip treatment of stone fruits in a suspension of *Bacillus subtilis* controls the brown rot disease. McLaughlin *et al.* (1992) observed reduction in the postharvest disease of grapes, caused by *Rhizopus stolonifer* and *B. cinerea*, when dipped in the suspension of the yeast *Candida guilliermondii* strain 87 (US-7).

Thus dusting with a talc based formulation of *T. harzianum* consisting of 25 per cent propagules can be considered as a method for the successful control the postharvest rot of brinjal caused by *P. vexans* and that of tomato by *G. candidum* and *R. solani*.

SUMMARY

6. SUMMARY

The fungi found associated with the postharvest spoilage of brinjal, chilli and tomato were isolated. *Phomopsis vexans*, *Phytophthora* sp. and *Pythium aphanidermatum* were found associated with postharvest rot of brinjal. *P. vexans* was most frequently isolated and hence studied in detail. In chilli, *Colletotrichum capsici*, *Phytophthora capsici*, *Alternaria alternata* and *Botrytis cinerea* were found associated with postharvest diseases of which *C. capsici* was most frequently isolated and hence studied in detail.

In tomato, *Geotrichum candidum*, *Rhizoctonia solani*, *Fusarium solani*, *Rhizopus stolonifer*, *Pythium aphanidermatum* and *Alternaria alternata* were found associated. *G. candidum*, *R. solani* and *F. solani* were frequently isolated and hence studied in detail.

The epiphytic mycoflora of the brinjal, chilli and tomato fruits was isolated. Brinjal yielded maximum number of epiphytes (18) followed by tomato (14) and chilli (12). All the epiphytes were subjected to dual culture and the interaction types recorded. Based on the dual culture tests *Gliocladium virens* and *Trichoderma harzianum* isolated from the surface of brinjal, were found to be the best in inhibiting the important pathogens of chilli, brinjal and tomato.

G. virens was found to inhibit *P. vexans* and *C. capsici* by coiling and vacuolation while with *G. candidum* disintegration of mycelia was same. *R. solani* was inhibited by granulation of mycelia. G. *virens* was observed to inhibit *F. solani* by the mechanism of coiling and penetration.

The mechanism of inhibition was also studied in detail with *T. harzianum*. *C. capsici* was inhibited by formation of infection pegs while with *P. vexans* and *R. solani* by coiling and vacuolation and with *G. candidum* disintegration of mycelia was observed. *F. solani* was found to be inhibited by the mechanism coiling and penetration by *T. harzianum*.

In vivo testing of *G. virens* and *T. harzianum* showed that *G. virens* could cause rotting of tomatoes while *T. harzianum* was observed as a good antagonist against the pathogens of brinjal, chilli and tomato.

Four carrier materials *viz*., coirpith, wheat flour, charcoal and talc were tested for their efficacy to support the growth of antagonistic fungi. The conidial count and its germination percentage were maximum in talc, when compared to the other carrier materials.

In a 25 per cent formulation of *T. harzianum*, the product was found to show 1.1×10^6 spores /g of the formulation at the end of 42 days of storage at room temperature.

On comparing the various methods of application, both, dusting and dipping were found to be effective in controlling the infection by *P. vexans* on brinjal. In tomato dusting was found to be effective in controlling infections by *G. candidum* and *R. solani*. The formulation was not affected in controlling the storage rot of chillies caused by *C. capsici*.

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* Original not seen

APPENDIX

APPENDIX - I
Culture media used

Potato dextrose agar (PDA)

Potato	:	200 g
Dextrose	:	20 g
Agar	:	20 g
Water	:	1000 ml

Martin's media with rosebengal and streptomycin

Dextrose	:	10.0 g
Peptone	:	5.0 g
Potassium dihydrogen phosphate ($KH_2 PO_4$)	:	1.0 g
Magnesium sulphate ($MgSO_4 7H_2O$)	:	0.5 g
Agar	:	20.0 g
Distilled water	:	1000 ml
Streptomycin sulphate	:	0.3 ml per 100 ml of cooled media
Rosebengal	:	1 part in 30,000 parts of the media
pH	:	6.8

BIOCONTROL OF POSTHARVEST DISEASES OF SOLANACEOUS VEGETABLES

By

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ABSTRACT OF THE THESIS
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ABSTRACT

The fungi commonly associated with the spoilage of solanaceous vegetables viz., brinjal, chilli and tomato under storage conditions were isolated and identified.

Based on the frequency of occurrence *Phomopsis vexans* was selected in brinjal, *Colletotrichum capsici* in chilli and *Fusarium solani, Rhizoctonia solani* and *Geotrichum candidum* in tomato. These fungi were used for the further study.

The studies on epiphytic mycoflora of the fruits yielded maximum epiphytes for brinjal followed by tomato and chilli. These included several fungi, yeast and many other saprophytes.

Yeasts like *Cryptococcus* sp. and *Sporobolomyces roseus* and fungi like *Aspergillus niger, Pencillium wortmanii* and *Fusarium solani* were common to all the three vegetables. All the epiphytes were subjected to *in vitro* studies and tested against each pathogen. *Gliocladium virens* and *Trichoderma harzianum* were found to be effective from the *in vitro* test.

The mechanism of *G. virens* and *T. harzianum* was studied in detail and was found to be through coiling and penetration, coiling and granulation, coiling and vacuolation, disintegration of mycelia and formation of infection pegs.

The role of *T. harzianum* and *G. virens* as biocontrol agents was studied *in vivo*. It was found that *T. harzianum* was effective in reducing the rots for brinjal in all the pathogens under study. *G. virens* however was found to infect the tomato fruits.

T. harzianum was formulated in talc, coir pith, charcoal and wheat flour. Among these, maximum viability was recorded in talc. The number of spores increased initially only in the case of flour while for the rest it registered a steep decline and a stagnation after fifth week.

T. harzianum was thus formulated in talc at 25 per cent concentration.

The viable spore count at the end of the seventh week was of the order of 10^6 c.f.u./g of the formulation.

The formulated antagonist was applied on the fruit by dipping as well as dusting. For the chilli anthracnose (*C. capsici*) neither of the methods were effective. In the case of brinjal rot (*P. vexans*) dusting was as effective as dipping in controlling the rot, for tomato dusting was slightly better than dipping in controlling sour rot (*G. candidum*). Both the methods were ineffective in controlling *Fusarium* rot (*F. solani*) of tomato while *Rhizoctonia* rot was controlled better by dusting than dipping in the formulation of the antagonist.